Università
Ca'Foscari
Venezia

Scuola Dottorale di Ateneo
Graduate School

Dottorato di ricerca
In Scienze Chimiche
Ciclo XXVII
Anno di discussione 2015

STUDIO DI SISTEMI CATALITICI INNOVATIVI PER LA SINTESI DI PRODOTTI DELLA CHIMICA FINE

(Study of innovative catalytic systems for the synthesis of fine chemicals)

SETTORE SCIENTIFICO DISCIPLINARE DI AFFERENZA: CHIM/04
Tesi di Dottorato di Riccardo Tassini, matricola 816724

Coordinatore del Dottorato	**Tutore del Dottorando**
Prof. Maurizio Selva	**Prof. Stefano Paganelli**
	Co-tutore del Dottorando
	Prof. Oreste Piccolo

Summary

1. INTRODUCTION

1.1 The fine chemical industry

The term “fine chemicals” defines complex or single-pure substances, produced in limited quantities in multipurpose plants by multistep batch processes. Agrochemicals, pharmaceuticals, fragrances and other products can be include in this definition. The manufacture of fine chemicals is essentially described by the following features[1]:

Table 1.1. Typical features of fine chemical industry

Complex molecules (isomers, stereochemistry, several functional groups) with limited thermal stability.
Usually classical organic reactions are used, catalysis is an exception.
High purity requirements (usually >99%, <10 ppm metal residue and ee >98% in pharmaceuticals).
Small-medium scale productions (1–1000 t per year for pharmaceuticals, 500–10 000 t per year for agrochemicals).
Multi steps synthesis batch operations (5–10 steps for pharmaceuticals and 3–7 for agrochemicals).
Short-medium product live (often <20 years).
Short development time for the production process (<few months to 1–2 years) since time to market affects the profitability of the product.
Very severe specifications and production rules.
Intellectual properties (IP) constrains.
Large amounts of unwanted wastes are produced (solvents, salts, by-products, etc., that must be eventually recycled or discarded).

The above described characteristics, together with the wide range of products included in the definition of fine chemicals, make this area highly heterogeneous. This means that involved reactions, processes, solvents and auxiliaries are very different. In the field of fine chemicals, in particular for their production in western countries, it has become mandatory to pay attention to more efficient and sustainable productive processes, in agreement with the green chemistry principles.

1.2 Green Chemistry

Over the past twenty-five years, sensibility to the environment and human health has increased significantly. Some environmental disasters (Seveso 1976, Bhopal 1984) revealed that the mismanagement of the chemical processes and the use of harmful substances can lead to environmental pollution and depletion of natural resources. These bad choices and wrong management still have consequences for the population and the territory.

In the early ’90s, Anastas and Warner developed a new concept concerning chemistry and called it “green chemistry”[2]. The aim of green chemistry is to reduce the use and manufacture of hazardous substances in the design and synthesis of chemicals products. The philosophy of green chemistry is to achieve a more sustainable development with the lowest environmental impact. For this reason the concept of chemical process is changed. When a new process is designed, it is important to obtain the desired molecule by simple and efficient way, but it must also verify the impact of certain choices (auxiliaries, reagents, plant equipment, etc.) on the environment and human health. This concept is regulated by 12 new principles that should be followed to develop an economically and environmentally sustainable process:

Table 1.2. The twelve green chemistry principles[2]

1. It is better to prevent waste than to treat or clean up waste after is formed.
2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.
5. The use of auxiliary substances (e.g. solvents, separations agents, etc.) should be made unnecessary wherever possible and, innocuous when used.
6. Energy requirement should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be performed at ambient temperature and pressure.
7. A raw material of feedstock should be renewable rather than depleting wherever technically and economically practicable.
8. Unnecessary derivatization (blocking group, protection/de-protection, temporary modification of physical/chemical process) should be avoided whenever possible.
9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.
11. Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. Substance and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions and fires.

Although the concept of green chemistry is noble, it is mandatory to study the chemical processes from an industrial and economic point of view: so, the concept of "sustainable chemistry" was introduced. The term "sustainable chemistry" includes the philosophy of green chemistry, the economic issues and the effects of certain choices for present and future[3].

Normally, the quality of a reaction is determined by yield and selectivity, expressed as below:

$$yield\ of\ reaction = \frac{yield\ of\ product}{theoretical\ yield\ of\ product}$$

$$selectivity\ of\ reaction = \frac{yield\ of\ desired\ product}{amount\ of\ converted\ substrate}$$

However, these parameters do not highlight that in a synthesis all starting materials should be preferably present into the final product. In fact, for example, the use of solvents, different kinds of auxiliaries and the formation of waste are not considered. Other parameters, known as green metrics, were introduced with the green chemistry concept and are summarized in the Table 1.3[4]. These parameters may include the use of auxiliaries, solvents, the amount of produced wastes, etc..

Table 1.3. The most important green metrics

Green metrics	Description	Considerations
$\boldsymbol{Atom\ efficiency}: \dfrac{\boldsymbol{MW\ of\ desired\ product}}{\boldsymbol{MW\ of\ all\ reagents}}$	The atoms of the starting materials should be present also in the product.	Reaction yield, molar excess and used solvents are ignored.
$\boldsymbol{RME} = \dfrac{\boldsymbol{mass\ of\ product}}{\boldsymbol{\Sigma mass\ of\ reagents}}$	Reaction mass efficiency (RME) describes the amount of reagents that are converted into the product.	The use of solvent is not considered, but yield of reaction and molar excess of reagents are included.
$\boldsymbol{E} = \dfrac{\boldsymbol{weight\ of\ total\ waste}}{\boldsymbol{weight\ of\ desired\ product}}$	E-factor (E) is the ratio between the weight of total waste and the weight of desired product.	Yield of process, used solvents and amount of waste are considered.
$\boldsymbol{PMI} = \dfrac{\boldsymbol{weight\ of\ raw\ material\ input}}{\boldsymbol{weight\ of\ desired\ product\ out}}$ **From PMI is possible to calculate the E-factor:** $\boldsymbol{E = PMI - 1}$	Process mass intensity (PMI) defines the ratio between the quantity of raw materials input in the process and the quantity of obtained product.	PMI has been chosen as the most significant green metric value for evaluating the sustainability of production by American Chemical Society's Green Institute Pharmaceutical Roundtable.

In an ideal process, E parameter should be equal to zero but while for oil refinery and bulk chemistry processes this parameter is very close to one, for fine chemicals and pharmaceutical processes it may exceed 100 (Table 1.4)[5].

Table 1.4. E value for different industry segments

Industry segment	Product tonnage	E factor	Tonnage x E-factor
Oil refining	10^6-10^8	<1	<10^6-10^8
Bulk chemicals	10^4-10^6	<1-5	10^4-5x10^6
Fine chemicals	10^2-10^4	5-50	5x10^2-5x10^5
Pharmaceuticals	10-10^3	25->100	250-10^5

It is worth to note that for a correct estimate of the environmental impact, the volume of production must be considered.

The E factor increases from bulk chemicals to fine chemicals production. This fact can be explained by considering the typical features of fine chemistry and pharmaceutical industries reported in Table 1.1. As quoted before, products with very high purity are often required and sometimes there is a short time for the production process development; so the E-factor becomes high because large amounts of unwanted materials (solvents, salts, by-products, etc.) are obtained. In addition to the amount of wastes, it is also important to consider the waste impact on the environment. For this reason, another parameter was introduced. This parameter, called EIQ (environmental impact quotient), considers the intrinsic harmfulness of unwanted reaction products. This term (EIQ) is calculated by multiplying the factor E for an arbitrary term Q. The value of Q depends on the toxicity of waste, the disposal or recycling difficulty and its amount. Therefore, it is clear that the choice of a process depends not only on the quantity of used materials but also on the quality of wastes. The above mentioned green metrics do not consider this fact and for this reason different computing data bases have been developed. For example LCA (Life Cycle Analysis)[6]. represents the most comprehensive approach to determine the impact of human activities including chemical reactions. In this system all inputs and outputs associated with the investigated process are considered. It means that also energy requirement and the production method for each involved substance in the process are estimated. LCA is a complete approach to know all critical points of an investigated process, but it is important to note that this method involves many parameters. For this reason, the calculation could be difficult especially on laboratory scale. Other simpler methods applicable also on small scale have been so developed. EATOS (Environmental Assessment Tool for Organic Synthesis)[7] is a program that permits to analyze the impact of a reaction from different points of view. In fact, this system considers the amount of necessary reagents, the amount of waste produced for one Kg of desired product, the impact of reagents, products and wastes on the environment and human health. The full cost of process is also considered. Another interesting approach to investigate the impact of processes is the EcoScale method[8]. This computing system allows to obtain indications about on a reaction carried out, for example, on lab scale. This method considers six important reaction parameters: yield, costs and safety of used materials, equipment complexity, operation conditions, work-up and purification of product. A score ranging from 0 (ruinous reaction) to 100 (ideal reaction) can be assigned for each reaction. This score is assigned adopting the criteria reported in Table 1.5.

Table 1.5. Penalty points to evaluate the EcoScale

Parameters	Penalties
Yield	= [100-yield (%)]/2
Price of reaction components	
Inexpensive (<10 €)	0
Expensive (>10 € and <50 €)	3
Very Expensive (>50 €)	5
Safety*	
Common set-up	0
Controlled addition of chemicals	1
Unconventional activation techniques	2
Pressure equipment (>0.1 MPa)	3
Any additional special glassware	1
(Inert) gas atmosphere	1
Glove box	3
Temperature/Time	
Room temperature <1 h	0
Room temperature <24 h	1
Heating <1 h	2
Heating <24 h	3
Cooling to 0°C	4
Cooling <0°C	5
Workup and purification	
None	0
Cooling to room temperature	0
Adding solvent	0
Simple filtration	0
Removal of solvent bp <150 °C	0
Crystallization and filtration	1
Removal of solvent bp >150°C	2
Solid phase extraction	2
Distillation	3
Sublimation	3
Liquid-liquid extraction	3
Classical chromatography	10

*penalty is based on hazard warning symbols

Taking into account Table 1.5, the EcoScale of reaction is calculated in this way:

$$\mathit{EcoScale} = 100 - \mathit{sum\ of\ individual\ penalties}$$

An excellent reaction has a score >75, an acceptable reaction has a score >50 and an inadequate reaction has a score <50.

By considering the importance to use low-risk substances for human health and environment, to improve the safety and the economical aspect of a reaction, it is possible, for instance, to evaluate in details the use of solvents. In fact, they are intensively used in many of process steps (solvent of reaction, extraction and purification of products)[9]. Solvents are often unsafe for human health and environment and furthermore they can be difficult to recover and recycle. It would be better to avoid their use, by carrying out the reaction under neat conditions and finding the simplest procedure to recover the desired product. However, it is not always possible to perform the reaction without solvent for example if the reagents are in different phases or if it is necessary to dissipate heat. In these cases, it is suggested to use more eco-friendly and safe solvents. During this work of thesis, when possible, solvents with low environmental impact or reactions under neat conditions were investigated.

Another important aspect to verify for improving reaction efficiency is the development of a synthetic scheme where catalysts are used. A catalytic reaction is usually more selective (chemo-, regio- and/or stereo-selective) and it implies a reduction in the formation of by-products and lower purification steps. However some difficulties to replace stoichiometric reactions with catalytic alternatives may occour especially in fine chemicals industry; very active and selective catalysts are necessary and often there is not time (and money) to prepare new catalysts or to find the most suitable commercial catalytic materials. Furthermore, it is also important to remember that catalysts have, sometimes, good performances when the substrates are highly pure and this is not always possible, especially in industrial processes[10].

1.3 Catalysis: introduction and definitions

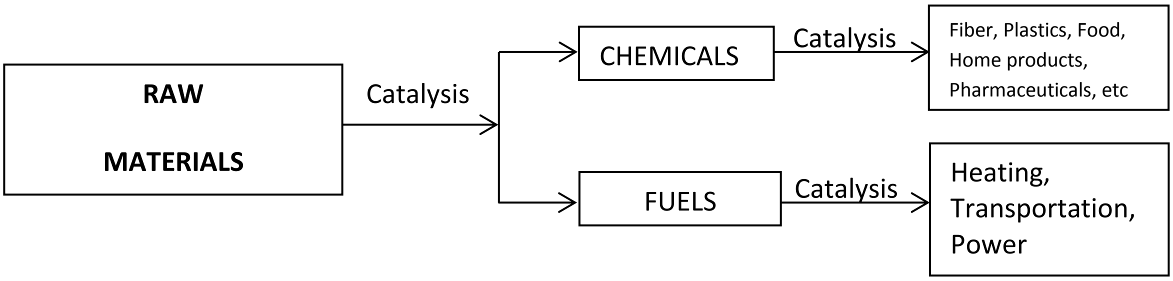
The term catalyst was introduced by Berzelius in 1836 to explain various transformations that happen in chemical reactions. He assumed that catalysts possess special powers that can influence the affinity of chemical substances[11]. A definition of catalyst that is still valid today is due to Ostwald (Nobel prize in chemistry in 1909) that in 1895 defined the catalyst as a substance that accelerates a chemical reaction without affecting the position of the equilibrium. In an ideal case, the catalyst should not be consumed during the reaction, but in practice it may undergo chemical changes and its activity may become lower (catalyst deactivation). Besides the activity, other important properties of catalysts are selectivity and stability. Here are reported the definitions of activity, selectivity and stability of a catalyst:

1. **Activity**: which is the measure of the increased conversion rate of the substrate due to presence of the catalyst.
2. **Selectivity**: which expresses the fraction of starting material that is converted into the desired product; three different kinds of selectivity can be observed according IUPAC “Gold book”[12]: a) *regioselective reaction* is one in which one direction of bond making or breaking occurs preferentially over all other possible directions; b) *chemoselective reaction* is the preferential reaction of a chemical reagent with one of two or more different functional groups; *stereoselective reaction* is the preferential formation in a chemical reaction of one stereoisomer over another; when the stereoisomers are enantiomers, the phenomenon is called enantioselectivity and is quantitatively expressed by the enantiomeric excess (e.e); when they are diastereoisomers, it is called diastereoselectivity (d.i) and is quantitatively expressed by the diastereoisomeric excess (d.e).
3. **Stability**: which defines the lifetime of catalyst during the period in which it maintains its properties; causes of degradation may be mechanical or thermal phenomena or due to a chemical stress.

The last property is the most important. The catalysts that lose activity during a process can be sometimes regenerated but they have to be replaced after many regenerations. The total lifetime of a catalyst is important for the economics of the process[11].

1.4 Impact on the society[13]

Catalysis is very important in our modern technological society. The worldwide economy is based on catalytic production of chemicals and fuels.



Scheme 1.1. The use of catalysts in various steps of the chemical industry

Petroleum, power, chemicals and food industries, which account for more than 10 trillion $ of gross world product, are largely dependent on catalytic processes. In fact, catalysts technology is much used in chemical and petrochemical industry, two of the largest industries with worldwide sales. In the Table 1.6 the increasing trend of the use of catalysts in some industrial fields is shown.

Table 1.6. Catalyst sales (billions of US$)[a] in different chemical industry sectors

Category	1993	1997	2000	2001	2005	Annual grow rate %
Auto catalysts[b]	2,18	2,65	6,75	7,09	8,61	5,0
Chemicals						
Biocatalyzed processes and fermentation	1,79	2,26	2,69	2,83	3,44	6,0
Bulk	1,75	1,80	1,97	2,07	2,51	3,0
Fine-chemicals[c]	0,41	0,45	0,49	0,52	0,63	3,0
Polymers	0,94	1,70	2,02	2,12	2,58	5,0
Other sectors						
Enviromental	0,62	0,67	0,73	1,01	1,23	10,0
Petroleum refining	1,80	2,10	2,00	2,32	2,82	4,0
Recovery of spent catalysis	0,62	0,65	0,75	0,79	0,96	5,0
Total	**10,1**	**12,3**	**17,4**	**18,7**	**22,8**	**5,0**

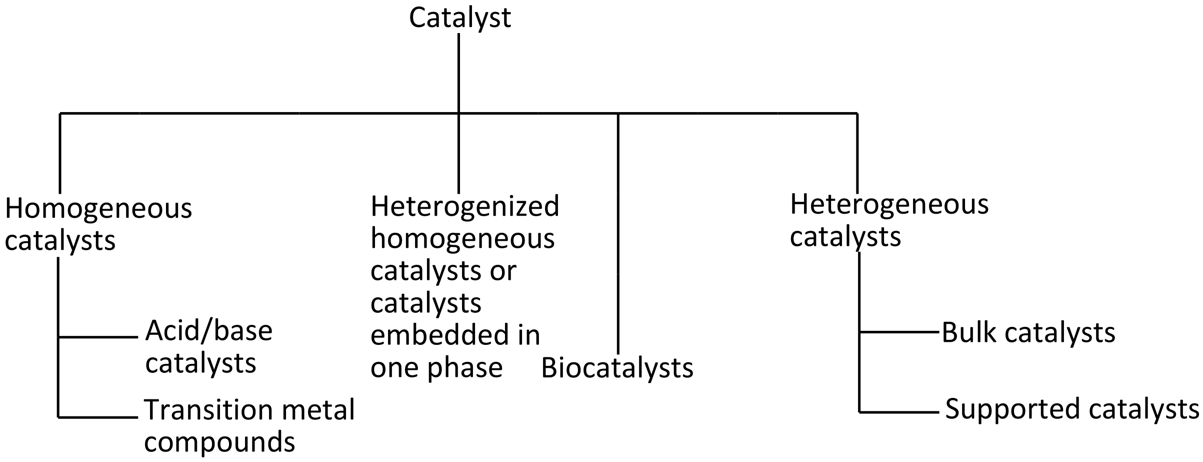
[a] It does not include about 1,2 billion of dollars per year of catalysts produced and consumed internally by chemical and petroleum companies, nor 4,3 billion of dollars per year in process/catalyst licenses and other royalties; [b] precious metal are included since 2000; [c] not including fermentation processes.

The use of catalysts is widely spread in different industrial segments especially in the environmental sector. Also the use of enzyme is growing and it is due to advances in biotechnology that permit to obtain them at reasonable price.

Finally, it is clear that the use of catalysts has not only an impact on chemical industry but also in the world economy.

1.5 Classification of Catalysts[11]

Catalysts can be classified according to the state of aggregation in which they act. They are devided in two large groups: heterogeneous and homogeneous catalysts. There are also intermediate forms: a) heterogenized (immobilized) homogeneous catalysts b) or catalytic species or confined into a different phase with respect to reagents and products.



Scheme 1.2. Catalyst classification

1.5.1 Heterogeneous and homogeneous catalysis

Heterogeneous catalysis refers to catalysts that are in a different phase with respect to reactants and products and normally it refers to the use of solid catalysts and liquid or gaseous reagents. This feature is very important because the separation from products and unreacted starting materials is simple. For this reason, different types of reactors have been developed. In many cases heterogeneous catalysts are confined into the reactor (fixed bed reactors) or in part of it, but in other cases they are suspended into the reactor (fluid bed reactors) and are filtered outside and sent back to the reactor. A typical heterogeneous catalyst is usually made by three components: an active catalytic phase, one or more promoters and the support. The active catalytic phase is usually made by transition metals (or their oxides, sulfides, carbides, etc.) normally dispersed into the pores of the support or on its surface[13].

The function of promoters depends on their nature: the texture promoters are used to facilitate the preparation and dispersion of the active phase and to maintain it in a well-dispersed state during the reaction conditions. The chemical promoters are additives that improve activity or selectivity of the catalytic phase. The role of the support is to facilitate the dispersion of the active catalytic phase and to stabilize it; indeed the heterogeneous catalysis is often affected by leaching of metal from support due to the reaction conditions. Reabsorption of metallic particles on the support may occur but very often after an aggregation and change of particle size[14].

There are many types of supports, characterized by surface area, pore volume and pore diameters. These features affect the dispersion and stability of the catalytic phase[13].

Table 1.7. Characteristics of several most common supports

Support/Catalyst	BET area (m^2/g)	Pore volume (cm^3/g)	Pore diameter (nm)
Activated carbon	500-1500	0.6-0.8	0.6-2
Zeolites (molecular sieves)	500-1000	0.5-0.8	0.4-1.8
Silica gels	200-600	0.4	3-20
Activated clays	150-225	0.4-0.52	20
Activated Al_2O_3	100-300	0.4-0.5	6-40
Kieselguhr (“celite 296”)	4.2	1.14	2200

Heterogeneous catalysts were the first to be used commercially and still now dominate the industrial practice. However, in the early 1960s homogeneous catalysis began to be applied profitably in many fields of industrial chemistry including the production of bulk and specialty chemicals, polymers, pharmaceuticals and food products. Currently the homogeneous catalysis is used in about one-third of catalyzed chemical processes and its use is growing especially for high-value fine chemicals and pharmaceuticals, where higher selectivity is required[15]. The homogeneous catalysis is referred to a process in which all reagents and catalyst are in the same phase during the reaction. Homogeneous catalysts may be small molecules or ions, such as HF, piperidine, metal ions, or complex molecules including metal-ligand or organometallic complexes, heteropolyacids or enzymes, which are soluble in the reacting fluid phase. The main differences between heterogeneous and homogeneous catalysts are shown in Table 1.8[13].

Table 1.8. Comparison between homogeneous and heterogeneous catalysts

Catalyst properties	Homogeneous	Heterogeneous
Active centers/site	Monodisperse atoms or molecules	Only surface atoms or molecules
Concentration	Low	High
Selectivity	High	Moderate to low
Diffusion problems/resistance to mass transfer	Practically absent	Present (mass-transfer-controlled reactions are common)
Reactions conditions	Mild (50-200°C)	Severe (often >250°C)
Applicability	Limited	Wide
Activity loss	Irreversible reaction with products (cluster formation); poisoning	Sintering of metal crystallites, poisoning, etc.
Structure/stoichiometric	Defined	Undefined
Modification possibilities	High	Low
Thermal stability	Low	High
Catalysis-product separation	Sometimes laborious (chemical decomposition, distillation, extraction)	Unnecessary for fixed-bed, filtration for fluidized bed
Catalyst recycling	Possible, but difficult	Unnecessary (fixed-bed) or easy (fluidized bed)
Cost of catalyst losses	High	Low

The major advantage of homogeneous catalysis toward heterogeneous catalysis is better activity and selectivity. This characteristic is often due to a higher chemical and geometric complexity of the homogeneous catalyst. It can have multiple valence states, form either ionic or covalent bonds and assume a great variety of geometric structures (for example planar, octahedral, trigonal etc.)[13]. However the main disadvantages of homogenous catalysts are their thermal instability and difficulty to separate them from products, solvent and unreacted substrates. This is the most limiting factor in a homogeneous catalytic process, especially when precious metals are used.

Table 1.9. Prices of some metals used in industry[16]

Metal	Price (€/Kg)
Au	31320
Co	32.90
Cu	7.00
Fe	1.00
In	700
Ga	275
Ni	18.80
Rh	37800
Pd	23100
Pt	39880

At the light of data reported in Table 1.9 appears fundamental to design efficient and easily recoverable catalysts where the losses of metal (especial precious metal) are minimized. Another approach would be to use cheaper metals rather than precious metals. However, it is important to consider that the efficiency sometimes is not the same. For instance, in the hydroformylation reaction expensive rhodium or cheapest cobalt complexes can be used as catalysts, but rhodium species allow to obtain higher yields and selectivities, under milder conditions[11].

Another problem associated with the use of metal species is their possible contamination of the final product. For example, in the synthesis of active pharmaceutical ingredients (APIs) the regulatory requirements set the upper limit of traces of some metals in the order of ppms (part per millions)[17].

Table 1.10. Comparative toxicological classification of the metals

Class 1 (toxic)	Class 2 (less toxic)	Class 3 (non-toxic)
Pt, Pd, Ir, Rh, Ru, Os, Mo, Ni, Cr, V	Cu, Mn, Ti, Sc	Zn, Fe

To remove traces of metals a tedious workup may be required and it becomes a costly affair due to the material loss. Also for this reason, it is important to develop very stable catalysts. During the development of a synthetic pathway it is so essential to assess either economic issues or regulatory aspects related to possible toxic metal contaminations. The separation and total recovery of the catalyst is a very limiting factor for the application of homogeneous catalysis in industrial reactions; so, in order to make them more applicable in productive processes, two major technologies for the recovery of homogeneous catalysts have been developed.

Table 1.11. Different technologies for heterogenization of homogeneous catalysts

Type of catalyst	Description	Advantages	Disadvantages
Immobilized (homogeneous) catalyst	Homogeneous catalysts are linked to different types of supports (polymer, carbon, ceramic, etc)	The catalyst is recovered from reaction mixture by filtration or decantation	Diffusional resistance; metal leaching
Embedded catalyst in a single phase	The reaction is carried out into two different phases and the catalyst is confined in one of them	At the end of the reaction, the two phases are separated and the catalytic phase is recovered	Difficult tuning of solvent mixture and suitable ligands. Sometimes metal and/or full may transfer partially or totally into the other phase.

To perform the reaction in biphasic system, different kinds of solvents able to confine catalytic species are ionic liquid, perfluorinated compounds, supercritical fluids and water have to be considered[18]. Doubtless, water is the most sustainable solvent as it presents the following properties:

1. It is not toxic;
2. it is not flammable;
3. it is polar and simple to separate from many organic solvents;
4. it is inexpensive and available in large quantity;
5. its density is different from many organic solvents;
6. gases dissolve well in it;
7. it has amphoteric behavior;
8. contaminations are easily recognizable.

To use this solvent, it is necessary to design a hydrophilic catalyst or a catalyst having hydrophilic ligands such as for example sulphonated phosphines. The application of this type of catalysts in industrial hydroformylation processes represents one of the most striking example of this catalytic methodology[19]. Other ligands with hydrophilic groups (SO_3^-; COO^-; NMe_3^+; OH^-;etc.) may be also used[20].

1.5.2 Acid Catalysis

The acid catalyzed processes play a key role not only in the petrochemical and bulk industry but also in the manufacture of a wide variety of fine and specialty chemicals including pharmaceuticals, agrochemicals, flavors and fragrances. The use of traditional Brønsted acids (H_2SO_4, HF, HCl, p-toluenesulfonic acid etc) or Lewis acids, such as $AlCl_3$, $ZnCl_2$, BF_3, is involved in many different processes especially for their cheapness[21]. However, the main problems of their use are the low selectivity and stability at high temperature and the generation of inorganic salts during the work-up due to the neutralization of crude reaction mixtures. This fact leads to the production of large amounts of aqueous wastes. A solution to this problem could be the replacement of traditional acid catalysts with recyclable solids acids. Many different heterogeneous acid catalysts have been developed; zeolites, heteropolyacids, supported metal oxides, clays and sulfonated perfluoro copolymers are widely used as Brønsted and Lewis acid in different types of reactions[22]. This choice has many advantages:

- Separation and recycling is facilitated;
- often solids acids are safer and easier to handle than their traditional counterparts, (e.g. HCl, H_2SO_4, HF);
- some traditional acids systems are highly corrosive and require expensive reactors and equipment;
- product contamination by traces of (neutralized) catalyst is generally avoided when the latter is solid.

In the context of acid catalysis it is also important to quote the use of some metal triflates as Lewis acids in many different kinds of reaction instead of traditional catalysts[23]. They are able to work in not dried organic solvents or also in water and often in real catalytic rather than in stoichiometric amount. However, it is also important to note some disadvantages: for example, their preparation may require the use of expensive rare earths or precious metals; moreover, the recycling of these catalysts is not always possible.

1.5.3 Bio-catalysis: introduction[13]

In biocatalysis, enzymes, large macromolecular polypeptides synthetized by living organisms, and rybozimes, composed of RNA, are used[24]. Enzymes having molecular weight between 10^4-10^6 Da are characterized by a unique three-dimensional structure with a binding site or pocket which is chemically and geometrically compatible with a single substrate or group of chemically related reactants. It means that enzymes have the ability to recognize a particular functional group or a particular substrate. Other important features of enzymes are selectivity, high turnover rates [for example, 10-10000 molecules/(enzyme$\cdot$s^{-1}) compared to 1-10 s^{-1} or less for conventional heterogeneous catalysis] and ability to be used at very low concentration (10^{-5} to 10^{-10} mol/L).

In 1976, 1800 enzymes were known[25], now there are over 3000 enzymes that have been fully characterized and over 7000 enzymes are predicted to exist[26]. The enzyme production is based on the extraction from biological sources (prokaryotic and eukaryotic cells) and subsequent purification (usually by crystallization or chromatography) but very often crude inactive cells with their enzymes content are used. Enzymes can be used in a native free form or as immobilized heterogeneous catalysts by using different technique to support them (for example, by a covalent binding to a support, use of a cross-linking by a bifunctional agent, adsorption on a solid surface, entrapment in a gel or on a membrane of special reactors).

The application of enzymes in industrial processes has rapidly increased in the last 20 years leading to:

- Significant cost, materials and energy saving;
- more environmentally friendly processes;
- significant simplification of difficult synthetic routes in pharmaceutical and fine chemical processes.

For these reasons, the market and the interest for enzymes increase every year.

1.5.3.1 Industrial application of enzymes and biotechnology

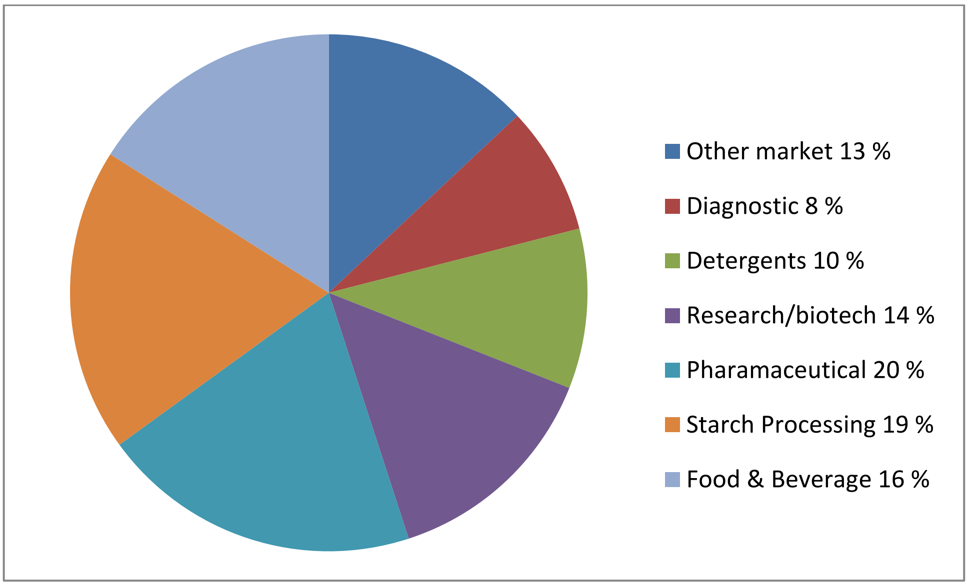
The term biocatalysis is referred to processes where biocatalysts are used. Precisely, the term biotransformation describes the process carried-out by whole non-living cells or the process performed by isolated enzymes with the addition of the reagents required for their activity. Fermentation describes on the contrary the process carried out by living natural, modified or recombinant microorganisms.

In the past 20-30 years biocatalytic processes have become more popular into the chemical industry: this is due to their high efficiency and selectivity. Another reason of their diffusion is the growth of biotechnology industry. This new industry is the result of several key scientific and engineering developments as:

1. the discovery and characterization of a large number of useful enzymes for industrial applications;
2. the development of techniques to engineer these enzymes in order to optimize their performance and stability; this has significantly increased the number of economically feasible industrial applications;
3. the development method for production, isolation, purification and immobilization which allows to strongly reduce the price of enzyme and the process;
4. the development of non-aqueous enzymatic processes that allows to expand the application of biocatalytic reactions;
5. the improvements of reactor technology which permits more accurate control of the enzymatic processes.

1.5.3.2 Economic impact of the use of enzymes in industry and future development

The estimated value of enzymes produced worldwide was roughly 2.5-3 billion US$ dollars in 2005 and is expected to grow at an average annual rate of 4-5 %[13]. It is difficult to estimate the corrent production of enzymes because there are many companies that produce their own catalysts for internal use. Graphic 1.1 summarizes the enzyme market for 2004 by industry:



Graphic 1.1. Use of enzymes in different industrial sectors

Other areas where the use of enzymes seems to faster expand are therapeutic biopharmaceuticals (for example treatment of cancer, multiple sclerosis etc.), analytical reagents and biosensors (for example clinical diagnosis, biochemical electrodes) and waste disposal and soil remediation.

Due to the characteristics as selectivity, activity and ability to work under milder conditions, enzymes are in perfect agreement with the philosophy of green chemistry. In view of this advantage the applications of enzymes are increased in the last decade and their use in industry is expected to continue.

The projection of growth in the use of enzymes is essentially due to the expectations that the production costs will be reduced and there will be some progress in different areas of research as:

1. conventional catalysis could be replaced by enzymes with higher selectivity in order to avoid costly product/side-product separations and disposal of undesirable waste products; enzymatic processes are also less energy intensive and often sustainable and it may be the push towards a greater use of enzymes in the future;
2. enzymes have begun to be used in the production of fine chemicals; their use will grow much in next years also thanks to the possibility to perfom biocatalyzed reaction in organic solvents and with unnatural substrates[27,28];
3. enzyme engineering permits to improve their activity, selectivity and thermal stability;
4. development of coenzyme-regenerating or coenzyme-immobilizing systems can help the spread of use of enzymes requiring a cofactor[29]; the high cost and difficulty to regenerate coenzymes and cofactors often limit the use of some biocatalysts in industrial applications; in alternative the use of whole cells rather than isolated enzymes could help many synthetic application[30];
5. development of new methods to immobilize biocatalysts could permit to recycle the them and to reduce the cost of process; many techniques have been studied to immobilize biocatalysts (cells or enzymes)[31];
6. the application of enzymes in biphasic reaction system, for example in aqueous/organic solvent and the use of a membrane system can facilitate the separation of biocatalysts from substrates and products and improve their recycle[32,33];
7. the discovery of new enzymes, which can replace the current synthetic methodologies and allow new reactions, currently considered "impossible" or very difficult to carry out by using actual technologies, could increase the use of this type of catalysis in industry.

In conclusion, the development of new discoveries, new production technologies, recycling and immobilization of enzymes are the keys for the spread of enzymatic catalysis in industry.

1.6 Analysis of reactions applied in fine chemicals industry

The fine chemicals industry, as already reported, is a heterogeneous sector. In fact, in this class, different products, including agrochemicals, pharmaceuticals, fragrances, etc., are grouped. For this reason, different types of reactions are involved, so it is very difficult to analyze their impact in whole fine chemistry sector. However, analyzing only the medicinal chemistry segment it can be possible to evaluate the incidence of some reactions classes (Table 1.12, adapted by reference [34]).

Table 1.12. Occurrence rate of reactions in medicinal chemisty

Reaction type	N° of reactions	% of total
Heteroatom alkylation and arylation	1687	23.1
Acylation and related processes	1635	22.4
C-C bond formation	841	11.5
Heterocycle formation	601	8.2
Protections	225	3.1
Deprotections	1319	18.0
Reductions	406	5.6
Oxidations	110	1.5
Functional group interconversion	413	5.6
Functional group addition	78	1.1
Total	**7315**	**100**

Therefore, different types of reactions require large variety of catalysts, especially in C-C bond formation, reduction and oxidation reactions. In the present thesis, also on the base of experience and interest of the research group where this work was carried out, a selection of reactions was made and studied. In this context, different new homemade catalysts were prepared and used on a wide range of substrates to verify their potential application in the synthesis of some industrial products.

2. AIM OF THESIS

The aim of this work of thesis was the preparation and the study of different families of catalysts which present the following features:

1. versatility towards different substrates and in large variety of reactions;
2. high selectivity;
3. possibility of efficient recycling;
4. possibility to operate in neat conditions or in an aqueous environment;
5. easy and reproducible preparation.

Therefore, the attention was focused on:

I. preparation, characterization and use of a heterogeneous Rh-based catalyst characterized by low metal content; in a described procedure[35], a very active 0.15 % Rh/Al_2O_3 was obtained in a simple way, exclusively used in the industrial hydrogenation process for the synthesis of the anti-inflammatory Nabumetone and not fully characterized; therefore, it was decided to prepare the same catalyst in order to verify the described procedure, to characterize it and use in other differ Rh-catalyzed reactions, so verifying its versatility, stability and recyclability;
II. preparation of new low metal content species, in particular Pd- and Pt-based alumina, in order to verify the possible extension of the procedure of point I toward other metals, then attempt to characterize them and check their potential catalytic activity in different types of reactions;
III. preparation of new bio-generated metal compounds using the microorganism *Klebsiella oxytoca* BAS-10, which is able to bind metal species on a particular exopolysaccharide (EPS) produced during the fermentation process; in research laboratory where this thesis was made, a Fe-based catalyst (Fe-EPS) had already been prepared and efficiently applied in the oxidation of phenol[36]; during this work of thesis, it was decided to prepare new mono and bi-metallic species such as Pd-EPS, Rh-EPS and Pd-Fe-EPS, by adding $Pd(NO_3)_2$, $RhCl_3$ or simultaneously $Pd(NO_3)_2$ and Fe citrate during the growth of the microorganism; the

characterization of these materials and evaluation of potential catalytic activity in different type of reactions was another aim;

IV. preparation of a new water soluble rhodium species by using a cheap ligand such as dihydrothioctic acid (DHTA) (Figure. 2.1);

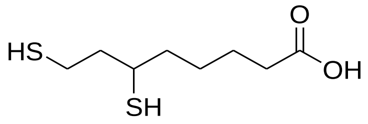


Figure 2.1. Dihydrothioctic acid (DHTA)

this acid, which presents two –SH groups, is able, in principle, to work as a bidentate ligand for the rhodium atom and moreover, the presence of another functional group, -COOH, in the molecule, when it is salified, might favour the solubility in water of the complex and also create a potential third binding site for Rh species;

V. in addition to metal-based catalysts, it was decided to investigate an ionomer with acid properties in order to verify its potential use in acid-catalyzed reactions. It was Aquivion®-PFSA (from here called Aquivion®), a perfluorinated ionomer commercialized by Solvay Specialty Polimers S.p.A. and currently used especially as membrane for electrolyte fuel cells; this ionomer presents sulfonic groups and therefore could be potentially used as a solid Brønsted acid catalyst instead of trifluoromethanesulfonic acid or other liquid traditional acids; furthermore, another goal of the present investigation was the check of the possible salification of this ionomer by direct treatment with metals, such as Fe, Ga and In, to prepare a potential heterogeneous Lewis acid usable in different reactions, such as Friedel-Crafts acylation and alkylation and, as catalyst, in $NaBH_4$ reductive amination;

VI. besides the preparation and the study of the activity of new metal based species, in this present research work it was proposed to study a method for the production and purification of a not commercially available carboxyreductase (CAR), known to selectively reduce a benzoic acid and some fatty acids into the corresponding aldehyde; this enzyme, originally obtained from *Micobacterium marinum*, was recovered by fermentation of genetically modified *Escherichia coli*[37], during this work of thesis it was decided to test also the activity of this enzyme in the selective reduction of 3-phenylpropionic acid to 3-phenylpropanal, reaction which could be interesting for a potential synthesis of a natural fragrance.

Another important goal of this thesis work was the potential application of the new home-made catalytic species to obtain chemicals of industrial interest comparing their performance with some commercially available catalysts (Table 2.1). In this context, it was also decided to investigate in some details the possible synthesis of Lioral^{TM} and Helional^{TM}, two valuable commercial fragrances.

Table 2.1. Reactions investigated during this thesis work

Reaction	Type of catalyst	Investigated catalyst
Hydrogenation of α-β unsaturated carbonyl compounds	Heterogeneous and biogenerated catalyst	0.28 % Pd/Al_2O_3;1.7 % Pd-EPS; 13 % Pd-EPS; 8.4 % Pd-Fe EPS
Hydrodechlorination		
Synthesis of β-ketoester by carbonylation		
Synthesis of aldehyde by carbonylation		
Reduction of nitro group	Heterogeneous and biogenerated catalyst	0.28 % Pd/Al_2O_3; 1.7 % Pd-EPS; 8.4 % Pd-Fe EPS; 0.27 % Pt/Al_2O_3
Hydroformylation	Heterogeneous, bio-generated and hydrosoluble catalyst	1.2 % Rh-EPS; 0.18 % Rh/Al_2O_3; RhDHTA
Reduction of Phenol	Heterogeneous catalyst	0.18 %Rh/Al_2O_3; 0.28 % Pd/Al_2O_3
Reduction of a carboxylic group to the corresponding aldehyde	Enzyme	Carboxyreductase (CAR)
Friedel Crafts acylation and alkylation of aromatic and heteroaromatic compounds	Solid Lewis and Bronsted acid	Aquivion® and its saline species (Fe, Ga, In)
Reductive amination with hydrides		
Etherification, esterification, amidation		

3. DISCUSSION AND RESULTS

3.1 Preparation and characterization of the catalysts

In this paragraph, preparation and characterization of all heterogeneous-, biogenerated- and water soluble-catalysts are reported. Enzyme carboxyreductase and the Aquivion®–based acid catalysts are described in Chapter 3 (paragraph 3.11 and 3.12, respectively). The activities of all catalysts were investigated on different types of reaction and the obtained results are presented in dedicated paragraphs of Chapter 3.

3.1.1 Preparation of low content metal heterogeneous catalysts by deposition on an inorganic support (Al_2O_3)

Nowadays there are a lot of available heterogeneous catalysts but often they present some drawbacks. As a matter of fact, their performances (selectivity, activity, productivity, stability) can be unsatisfactory and they show good activity often with specific and highly pure substrates. For these reasons sometimes it is very difficult to find a suitable catalyst for the investigated process[10]. Furthermore, the heterogeneous catalysts may be often affected by metal leaching[10]. If this phenomenon is relevant, the catalyst cannot be recycled, so the biggest advantage of the use of heterogeneous catalysts is lost; finally, as also reported in Chapter 1, metal residues in the final products may be a problem especially if the used metals are toxic.

In the literature many procedures to obtain heterogeneous catalysts are reported[13]. Considering only the methodologies of metal deposition on the solid support (the support preparation is not object of this thesis work) it must be remembered that there are three general approaches:

1. deposition of the active metal component on a carrier by impregnation, adsorption, ion-exchange or precipitation followed by washing, drying, calcination and activation;
2. precipitation of a metal oxide or metal carbonate followed by drying, calcination and activation;
3. preparation of a dense, nonporous compound or alloy containing the active component, followed by extraction of the inactive material so leaving a porous, high surface area active phase.

The impregnation is the simplest and probably the most common procedure for dispersing a catalytic species on a pre-dried support by incipient wetness with an aqueous or non-aqueous solution containing a salt (precursor) of the catalytic element or elements. There are two techniques for impregnating the support: the incipient wetness impregnation method and the wet method impregnation. In the first technique, the precursor salt is dissolved in a volume of solvent equal to the support pore volume, in the second method an excess of solvent is used.
As already reported in Chapter 1, there are many types of supports characterized by different properties that can play an important role during the deposition or during the reaction.

Alumina is a common commercial support because it has excellent thermal stability and wide range of chemical, physical and catalytic properties. Moreover, it also reported that alumina is good support because the absorption of organic compounds on this material is very low, so minimizing the mass balance problems[38]. The alumina family consists of more amorphous or crystalline structures, with different surface area (0.5-600 m^2/g), pore sizes, size distributions and surface acidity. The structure and properties of alumina depend on the preparation method, purity of alumina itself, dehydration and thermal treatment. For example, in Figure 3.1.1, some of the more well-known transformations, as a function of calcination temperature, are reported. The thermal treatment determines the final crystal structure and the chemical and physical properties[13].

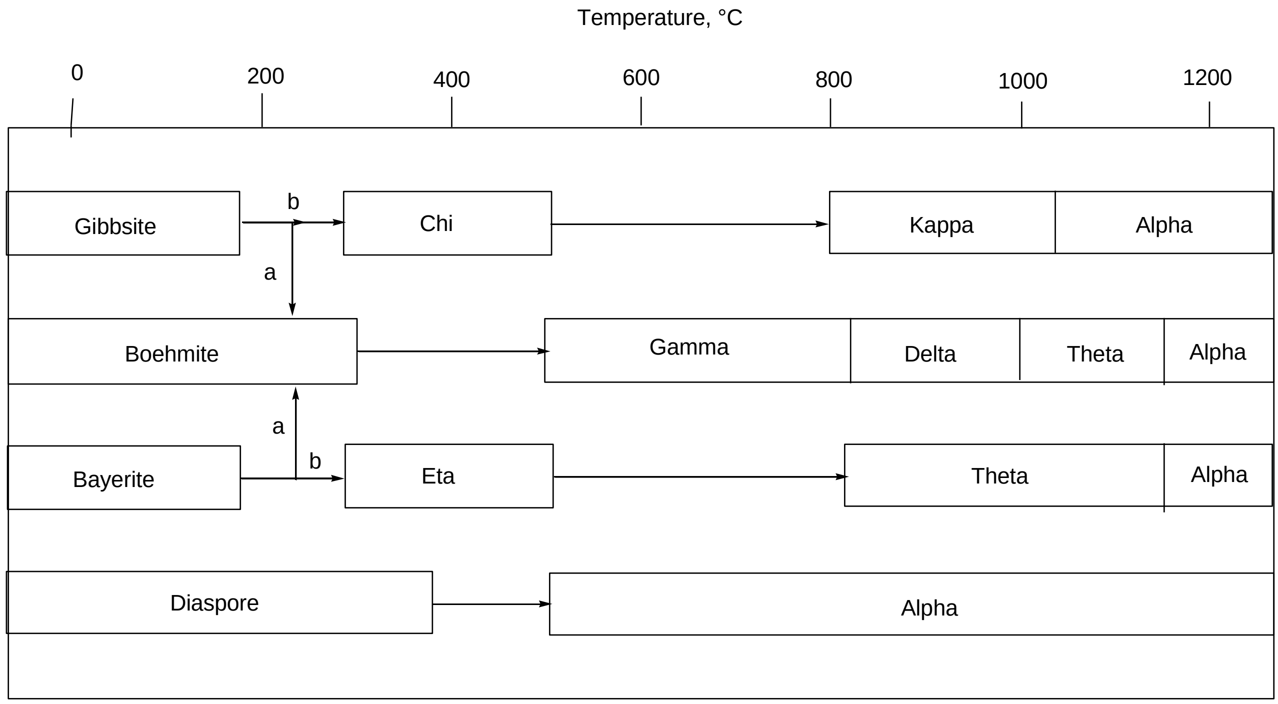


Figure 3.1.1. The different crystal structure of alumina in function of temperature; a) path favored for fine crystals, b) path favored for most moist or coarse particles

Table 3.1.1. Surface Area (SA), V_{pore} and D_{pore} of different types of Aluminas

T_{calc} (°C)	Alumina Phase	SA (m^2/g)	V_{pore} (cm^3/g)	D_{pore} (nm)
250	Pseudoboehmite	390	0.50	5.2
450	γ-alumina	335	0.53	6.4
650		226	0.55	9.8
850		167	0.58	14
950	δ-alumina	120	0.50	16.6
1050	θ-alumina	50	0.50	28
1200	α-alumina	1-5	---	---

In this thesis work, a preparation technique to obtain a low metal content catalyst, described into a patent[35], was replicated in order to obtain heterogeneous catalysts in a very simple way by application of a wet method. It is important to remark that the procedure was originally described only for the preparation of 0.15 % Rh/Al_2O_3; moreover, despite the industrial use of this catalyst in the reduction of a α-β-unsaturated ketone, both support and the catalyst obtained by metal deposition were not characterized until now.

3.1.1.1 General procedure for low metal content heterogeneous catalysts preparation

These catalysts were prepared starting from the salts of the desired metals (e.g. chlorides) which were reduced in the presence of hydrogen and trioctylamine (TOA) in an organic solvent (e.g. dried THF). Subsequently the impregnation of the metal particles was made by addition of this mixture on the support dispersed in the same dry organic solvent under H_2 atmosphere.

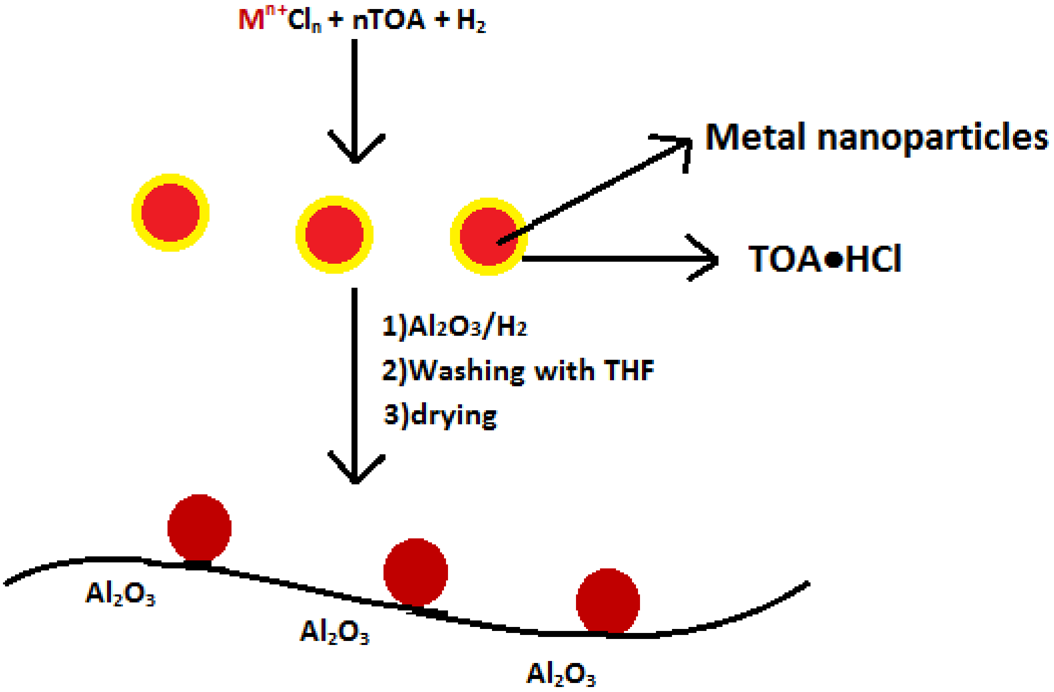


Figure 3.1.2. Preparation of heterogeneous catalyst.

After deposition of the metal on the support, the catalyst was filtered on a syntered glass filter and washed with dry THF, dried under vacuum at room temperature and can be immediately used without any calcination treatment. This is a clear advantage in comparison with known methods. The catalyst is stable and can be stored in the absence of moisture for months. The preparation is very simple and relatively fast and is performed under mild conditions. In fact, Rh, Pd and Pt catalysts were prepared by reduction of the metal salts for 3-24 h under 0.1-0.5 MPa of H_2 at 25°C and the subsequent deposition step carried out, under stirring, for 24 h in 0.1 MPa of H_2 atmosphere. An alternative procedure involves the contemporary reduction and metal particle deposition on the support. It is also important to emphasize that the procedure is potentially applicable to other metals; however necessary condition is that the metal salts are reducible under this experimental protocol.

3.1.1.2 Preparation and characterization of 0.18 % Rh/Al_2O_3 catalyst

Adopting the procedure reported in paragraph 3.1.1.1, Rh/Al_2O_3 was prepared. The amount of metal, determined by UNI EN 13674:2004 + EPA 6010C 2007 method, was 0.18 % with a 75 % yield compared to the theoretical metal amount.

The used alumina, defined as γ-alumina from the supplier's specifications, has the following characteristics (Table 3.1.2).

Table 3.1.2. Characteristics of used alumina according to the supplier

Characteristics	Value
Al_2O_3 content	99.5 %
Packed bulk density (PBD)	730 g/dm^3
Specific surface area (BET)	115 m^2/g
Particle size under 25 µm	0 %
Particle size under 45 µm	31 %
Particle size under 90 µm	93.5 %
Water content	0.32 %
Pore volume (water titration)	0.47 cm^3/g

The support was also analyzed by X-ray diffraction, BET adsorption, TEM microscopy and IR spectroscopy with pulses of CO, NH_3 and CO_2.

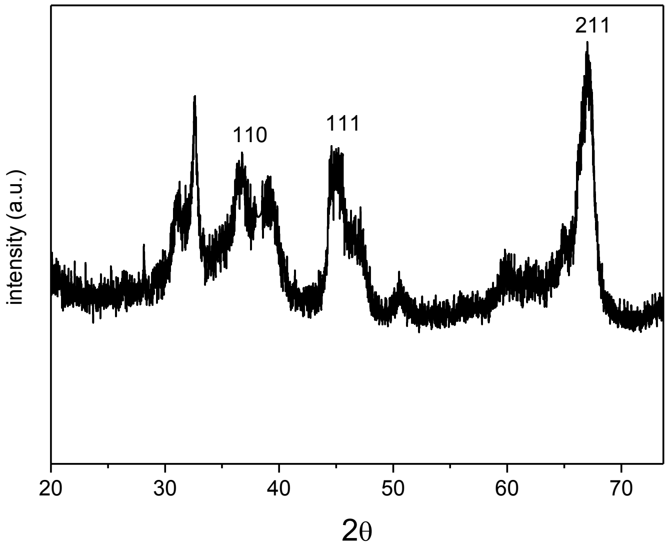


Figure 3.1.3. X-ray spectrum of used alumina

On the basis of the obtained refraction peaks, the support could be a γ-alumina in agreement with some literature[39] and with the supplier, but, on the basis of other literature data[40], the possibility of a δ-alumina could be perhaps more likely.

By BET analysis the specific area of the used alumina and of the obtained 0.18 % Rh/Al_2O_3 were determined and the hysteresis curves are reported in figure 3.1.4 (SSA is specific surface area):

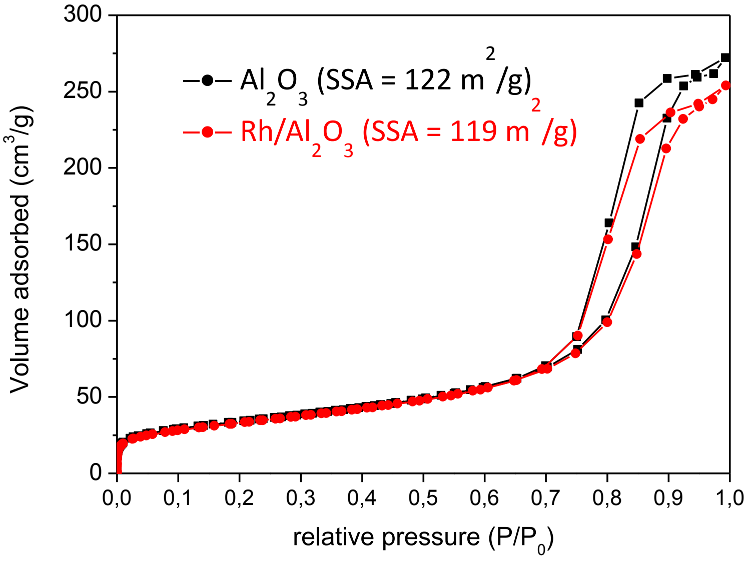


Figure 3.1.4. BET analysis of Al_2O_3 and Rh/Al_2O_3

The adsorption isotherms of N_2 on the alumin and on 0.18 % Rh/Al_2O_3 are of type IV: this means that both systems are mesoporous materials, characterized by calculated pores of 8-9 nm in diameter[13]. Furthermore, it was found that the specific surface area of the support is 122 m^2/g and this value is similar to that reported by the manufacturer; after rhodium deposition, this value slightly decreased to 119 m^2/g.

With impulse of NH_3 the presence of Lewis and Brønsted acidic sites, both on alumina (figure 3.1.5) and on 0.18 % Rh/Al_2O_3 (Figure 3.1.6), was determined.

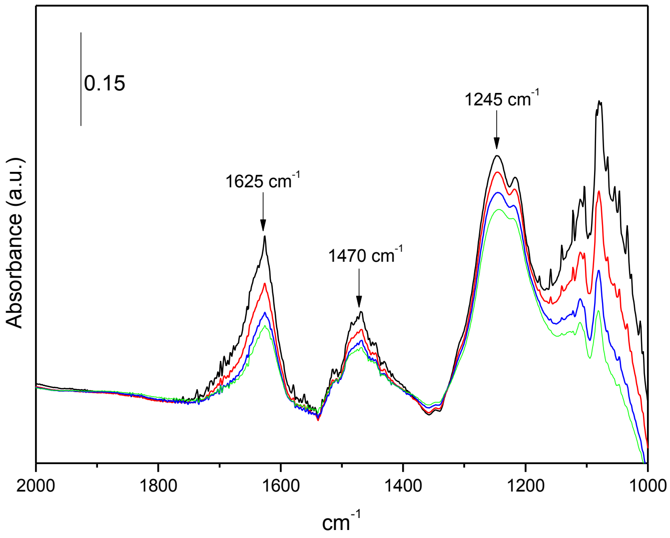


Figure 3.1.5. Adsorption of NH_3 on the used Alumina

The band at 1470 cm^{-1} which appears as a result of the adsorption of ammonia, is attributed to the bending vibration of ammonium ion. It reveals the presence of Brønsted acid sites on the surface of alumina. The other two bands at 1625 cm^{-1} and 1245 cm^{-1}, respectively, are due to bending vibrations of ammonia adsorbed in molecular form[41].

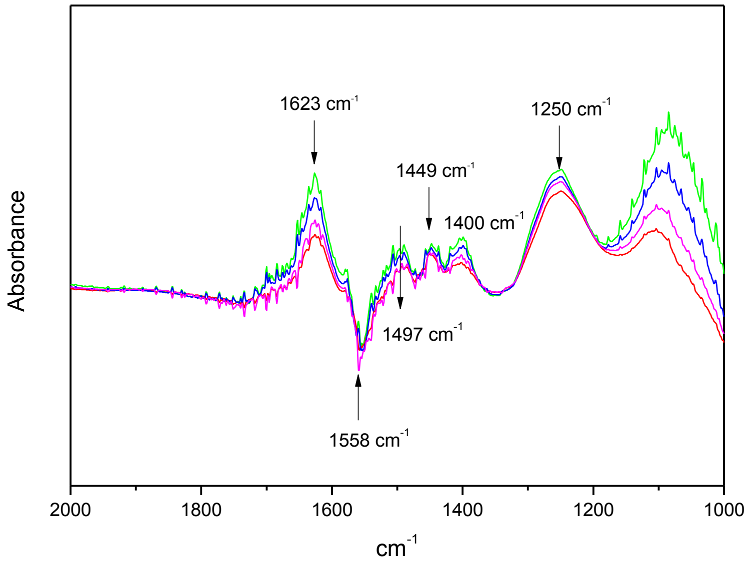


Figure 3.1.6. Absorption of NH_3 on Rh/Al_2O_3 0.18 %

The Brønsted acidity of the catalyst does not increase after Rh deposition and treatment with hydrogen, on the contrary it seems to become negligible. Furthermore, it is interesting to note that this sample also presents carbonate species on the surface (Figure 3.1.6, bands in the range 1600-1400 cm^{-1}). These species are probably formed by interaction of atmospheric CO_2 with the basic surface sites.

In order to determine the presence of the basic sites on the alumina a further analysis by CO_2 absorption was carried out. The CO_2 absorption on the support (Al_2O_3) reveals the formation of bicarbonate species (the three bands in the range 1800-1200 cm^{-1}) confirming the presence of basic sites (figure 3.1.7)[42].

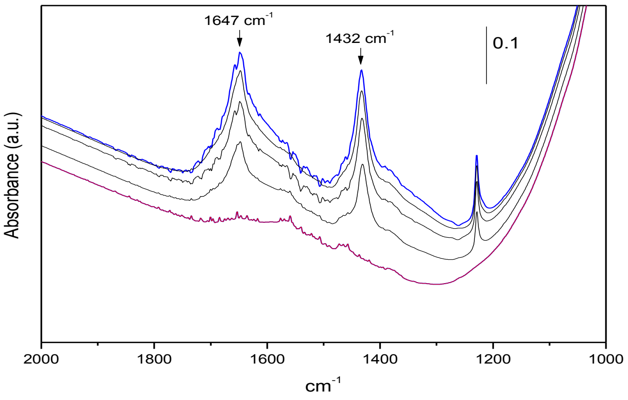
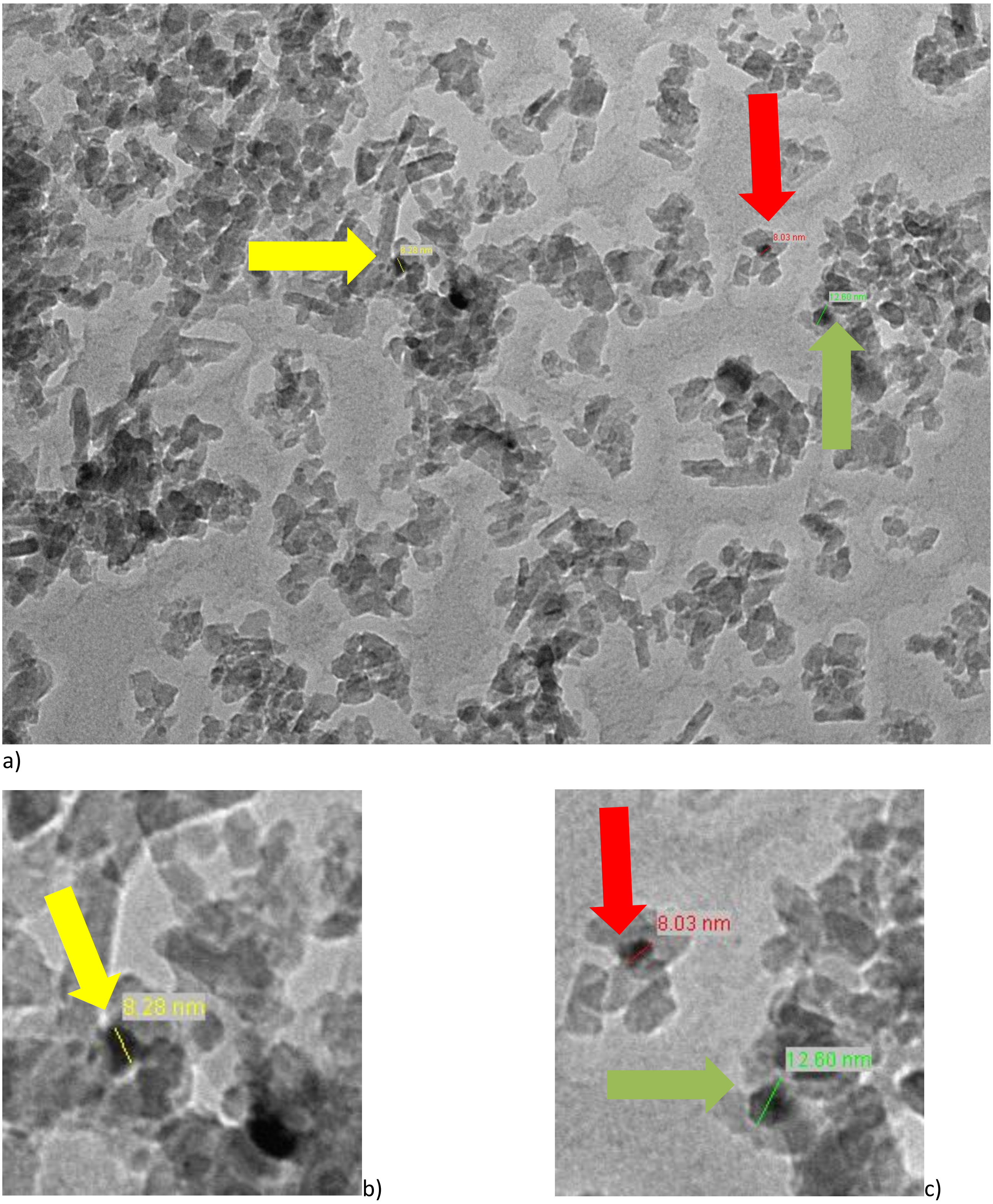


Figure 3.1.7. Absorption of CO_2 on Al_2O_3

The dimension of the Rh-particles was determined by TEM spectroscopy:



Yellow arrow: 8.28 nm; red arrow: 8.03 nm; green arrow: 12.60 nm.

Figure 3.1.8. TEM analysis of 0.18 % Rh/Al_2O_3: a) general imagine; b) and c) highlight on some clusters

The analysis shows that the size of rhodium clusters ranges between 8 and 13 nm, so confirming a nanostructure.

Finally, CO chemisorption analysis on 0.18 % Rh/Al_2O_3 was made after pre-treatment of the sample with H_2 by adopting the procedure reported at paragraph 5.13.7 of the experimental part:

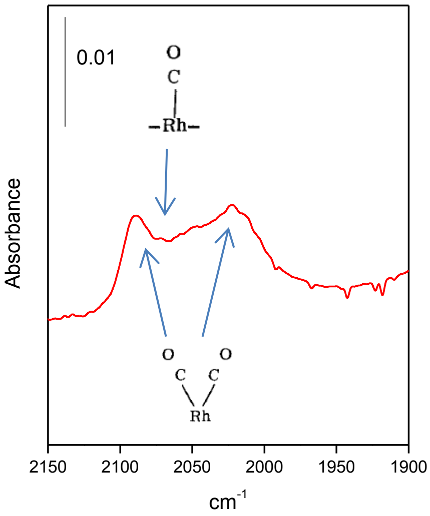


Figure 3.1.9. CO chemisorption analysis on 0.18 % Rh/Al_2O_3

It is possible to observe the formation of Rh-CO (band at 2050 cm^{-1}) and $Rh(CO)_2$ species (bands at 2086 cm^{-1} and 2015 cm^{-1}, respectively). The $Rh(CO)_2$ species are typical of big flat area or small rhodium clusters, including isolated rhodium atoms. Monocarbonyl rhodium species [Rh(CO)] are formed only in the presence of more active rhodium atoms and usually require the presence of larger rhodium aggregates[43,44].

3.1.1.3 Preparation and characterization of 0.28 % Pd/Al_2O_3 catalyst

Adopting the above described procedure, a new Pd-based catalyst was prepared. The amount of palladium (0.28%) was determined by atomic absorption after digestion of the sample in aqua regia, and a metal absorption higher than 93% was determined with respect to the theoretical amount. Due to the very low content of palladium, the CO chemisorption technique gave modest information: the presence of a very low band at 2085 cm^{-1}, reversible at room temperature, would seem to suggest the presence of a limited number of sites able to chemisorb CO; at the moment it was not possible to carry out further investigations with other techniques[45].

3.1.1.4 Preparation and characterization of 0.27 % Pt/Al_2O_3 catalyst

The catalyst was prepared following the above procedure. The amount of Platinum (0.27%) was determined by atomic absorption after digestion of the sample in aqua regia, and a metal absorption higher than 90% was determined with respect to the theoretical amount. The obtained catalyst is a white powder. The catalyst was analyzed by chemisorption of CO and at 2046 cm^{-1} it is possible to see the band due to the formation of Pt-CO linear monocarbonyl specie[45].

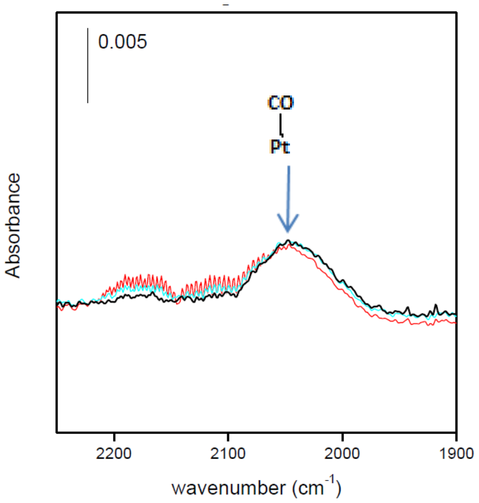


Figure 3.1.10. CO chemisorbtion on 0.27 % Pt/Al_2O_3

3.1.1.5 FT-IR analysis of three home-made catalysts

After pre-treatment with H_2, the three home-made catalysts on alumina were analyzed by FT-IR. From the obtained data reported in figure 3.1.11, it is possible to observe an enlarged band at about 3550 cm^{-1} due to hydroxyl groups forming hydrogen bonds. Furthermore, a band at 3000 cm^{-1} due to stretching of an aliphatic chain, and a band at 1700 cm^{-1}, due to the presence of carbonate species on the surface, are also detected.

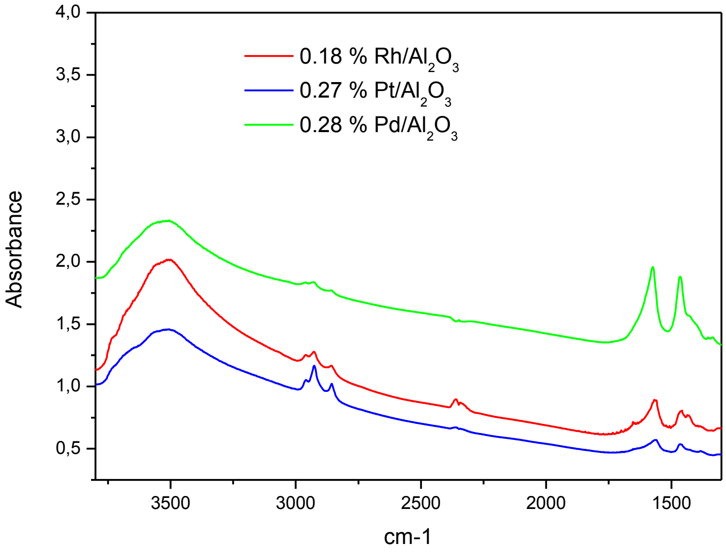
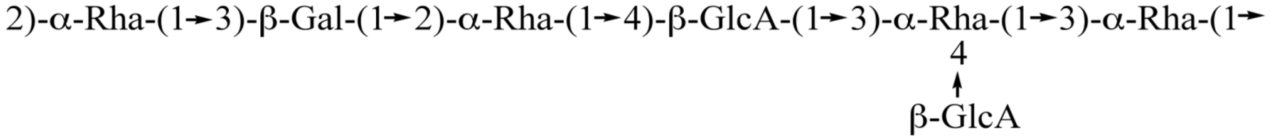


Figure 3.1.11. Analysis of three catalytic species by FT-IR

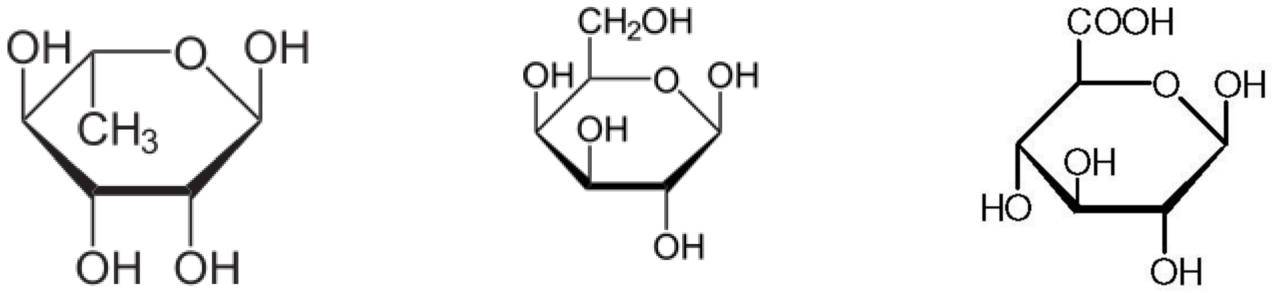
3.1.2 Biogenerated catalysts

A few years ago, a strain of *Klebsiella oxytoca* BAS-10, a facultative aerobic bacterium, was isolated from the acid drainage of a pyrite mine in Tuscany[46]. This microorganism is able to ferment citrate producing CO_2 and CH_3COOH. When the nutrients are finished and the stationary phase is started, the microorganism begins to produce and secret a peculiar polysaccharide (EPS), having a branched heptameric repeating structure with four α–rhamnose, two β-glucuronic acids and one α-galactose (Figures 3.1.12 and 3.1.13)[47].



$$2)\text{-}\alpha\text{-Rha-}(1\rightarrow 3)\text{-}\beta\text{-Gal-}(1\rightarrow 2)\text{-}\alpha\text{-Rha-}(1\rightarrow 4)\text{-}\beta\text{-GlcA-}(1\rightarrow 3)\text{-}\underset{\substack{4\\ \uparrow\\ \beta\text{-GlcA}}}{\alpha\text{-Rha}}\text{-}(1\rightarrow 3)\text{-}\alpha\text{-Rha-}(1\rightarrow$$

Figure 3.1.12. Sequence of monosaccharides that compose the heptamer repeat in unit present in the of the exopolysaccharide (EPS)



(Rha) **(Gal)** **(GlcA)**

Figure 3.1.13. Structure of ramnose (Rha), galactose (Gal) and glucuronic acid (GlcA)

When the microorganism grows in 50 mM ferric citrate instead of Na-citrate, it produces a thick Fe (III)-binding exopolysaccharide called Fe-EPS where EPS is able to bind particles of Fe (III) up to 36 % ± 4,5 %[48]. It is important to note that iron is a key nutrient for the cell but it becomes toxic at high concentrations. For this reason, the microorganism has developed a defensive system to deliver Fe (II) and Fe (III) ions outside the cell. The exopolysaccharide surrounds the cell and protects it from heavy metals, such as iron. This Fe-EPS complex was well characterized with different techniques[36,49].

By TEM and ESEM analysis various size nanoparticles of Iron were found randomly distributed surrounding cells. Furthermore, the presence of ordered layers of iron nanoparticles into Fe-EPS were observed by TEM micrographs[49].

By X-Ray analysis it was found that the bond between Iron and the exopolysaccharide is similar to the bond present in Ferritin. The atoms of metal are coordinated with six equidistant atoms of oxygen into the first sphere of coordination while in the second sphere there are six atoms of iron[49]. By XANES analysis it was found that Fe (II) and Fe (III) nanoparticles, with a medium valence of 2.82, are incorporated in EPS. The use of this bio-generated iron species (Fe-EPS) as promising catalyst in the hydroxylation of phenol, by using hydrogen peroxide, in an aqueous biphasic reaction medium as well as in pure water, was recently described[36]. At the light of these results it was thought to prepare new EPS species based on palladium or rhodium, two metals widely used as catalysts in various reactions of industrial interest.

3.1.2.1 Preparation and characterization of 1.7 % $(Pd\text{-}EPS)_{aerob}$ and activated 1.7 % $(Pd\text{-}EPS)_{aerob}$

Over the last decades, palladium nanoparticles with different composition, size, shape and distribution on support have been produced using different methodologies. Some approaches were found to obtain Pd(0) nanoparticles which were catalytically very active and more resistant to deactivation[50,51]. Nanoscale metal particles (NPs) are usually characterized by high surface area to volume ratios, high levels of stepped surfaces and high surface energies showing so improved reactivity[50,51]. Pd nanoparticles were obtained also using microorganisms. For instance, under controlled conditions, cultures of *S. oneidensis* are able to reduce Pd(II) to Pd(0) and to precipitate catalytic PdNPs, named ''bio-palladium'', associated with microbial cell surface[52,53]. This process might represent not only an economically interesting and eco-friendly production method to obtain nanoscale palladium, but the resulting "bio-palladium" was also found to be a promising catalyst towards reactions catalyzed by this metal. However, it is important to note that the so produced metal nanoparticles are associated to cell membrane. This fact could be a problem, for example, when these nanoparticles are used as catalyst to obtain commercial products. In fact, it is not possible to exclude traces of cells into the final product which could affect the health of the consumer. Therefore to obtain Pd species, embedded in a polysaccharide secreted out of the cells of *Klebsiella oxytoca* and easily recovered and purified, could be an innovative approach[54].

In order to replace iron with palladium on the same polysaccharide structure during cell growth of *Klebsiella oxytoca* strain BAS-10, it was necessary to adopt a new synthetic strategy and change the growing conditions of microorganism[47]. As a matter of fact, whereas Fe(III)-citrate is an important growth factor of *K. oxytoca*, as citrate is a carbon and energy source and iron an important micronutrient for the microorganism, Pd(II), also combined with citrate, is not a micronutrient is toxic and cannot support the cell growth, similarly to what was already demonstrated with Al(III)-citrate[55]. In order to circumvent the problem of palladium toxicity, the BAS-10 strain was grown aerobically in static mode in the presence of sodium citrate as sole carbon and energy source: in this way, an iron free EPS was produced. $Pd(NO_3)_2$ was then added to the culture, at the beginning of the stationary phase: after further 24 h of culture incubation a palladium species bound to EPS $(Pd\text{-}EPS)_{aerob}$ was extracted with 200 mg/L yield. The content of palladium (wt %) in $(Pd\text{-}EPS)_{aerob}$, determined by atomic absorption, was 1.7 %, therefore the amount of recovered Pd from colture is about 7 %. The structure of this biogenerated catalyst was determined by transmission electron microscopy (TEM), showing particles with diameters ranging from about 30 nm for the smallest ones up to 550 nm in the case of particles aggregation (Figure 3.1.14)[54].

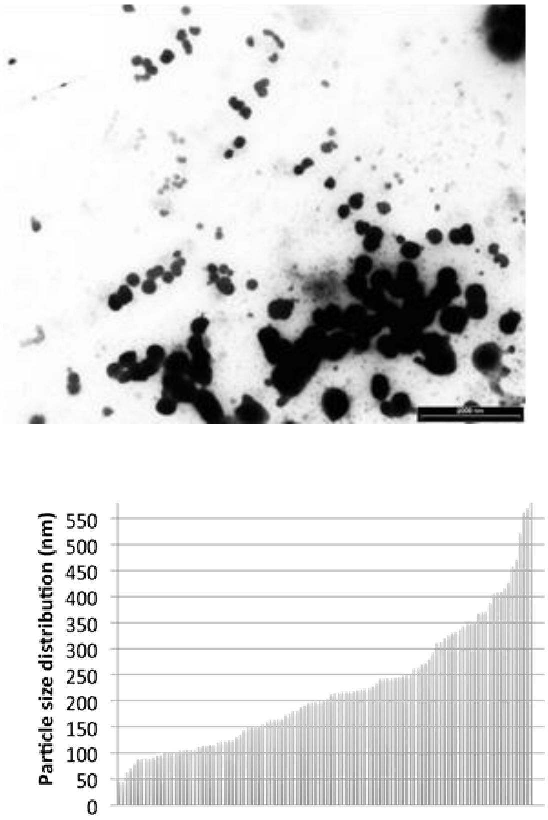


Figure 3 1.14. Micrograph at TEM of 1.7 % (Pd-EPS)$_{aerob}$

After a deep investigation on the different parameters that can influence the formation of 1.7 % (Pd-EPS)$_{aerob}$ better conditions to obtain this palladium species in a reproducible way was obtained; indeed, the growing conditions of *Klebsiella* are crucial to determine particle sizes and other specifications.

In order to verify the possible oxidation states of Pd ions, 1.7 % (PdEPS)$_{aerob}$ was analysed by X ray photoelectron spectroscopy (XPS) (Figure 3.1.15). The Pd 3d region of Pd-EPS exhibited two peaks due to Pd 3d(5/2) and Pd 3d(3/2). The Pd 3d (5/2) and Pd 3d(3/2) binding energies were obtained as 337.7 eV and 343 eV, respectively[54]. These binding energies are very close to those reported for Pd(II) ions[56,57]. Besides calcium, clearly evident in the spectrum (Figure 3.1.15), other elements as Mg (44.6%), P (38.9%) and N (15.3%) were detected in the sample.

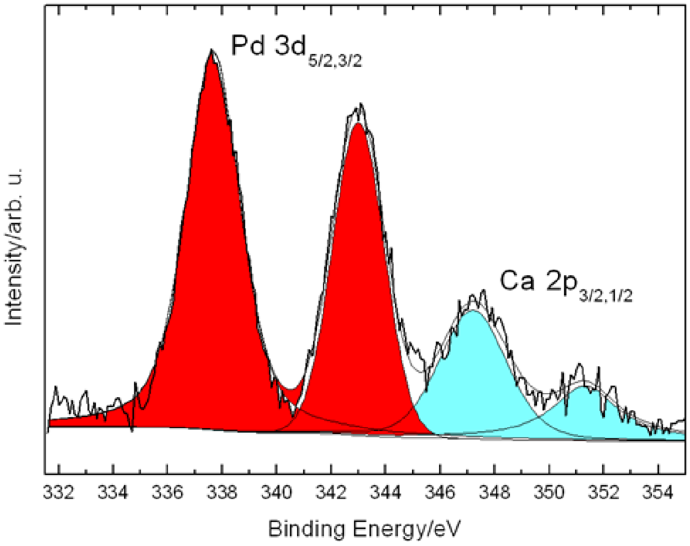


Figure 3.1.15. XPS spectrum of 1.7 % $(Pd\text{-}EPS)_{aerob}$

Since in reactions as hydrogenation, the catalytically active species is Pd (0), a new species called (activated 1.7 % $Pd\text{-}EPS)_{aerob}$ was obtained by treatment of an aqueous suspension of 1.7 % $(Pd\text{-}EPS)_{aerob}$ with 1 MPa of H_2 at 30°C for 21 h. While 1.7 % $(Pd\text{-}EPS)_{aerob}$ is a light yellow color, the activated form is gray, likely due to the presence of Pd (0)[54].

A TEM analysis of this new species showed (Figure 3.1.16) that the particles present in the original 1.7 % $(Pd\text{-}EPS)_{aerob}$ species tended to aggregate to form jagged undefined structures. It is known that biological nanoparticles are often not mono-dispersible and tend to cluster due to the capped polymeric molecule[53].

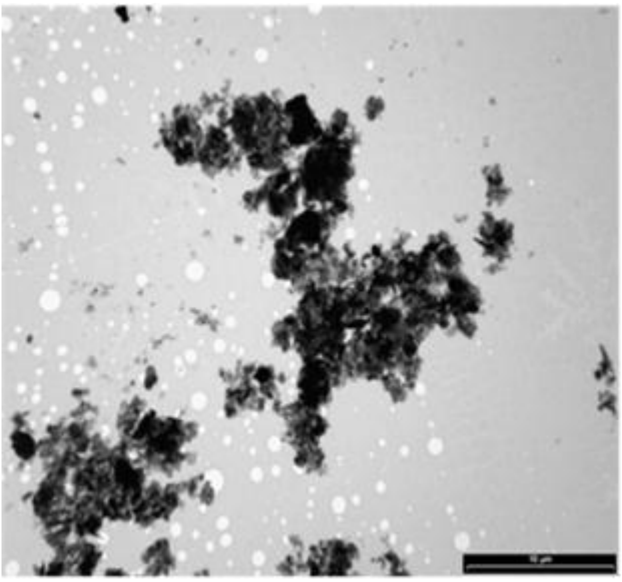


Figure 3.1.16 Micrograph at TEM of activated 1.7 % $(Pd\text{-}EPS)_{aerob}$

Also this new catalytic specie was analyzed by using XPS technique. The XPS analyses of "activated 1.7 % $(Pd\text{-}EPS)_{aerob}$ showed, besides the two peaks attributed to Pd(II) ions, two more peaks in the Pd 3d region with binding energies at 335.2 eV and 340.8 ev, respectively, very close to the values reported for Pd(0)[56,57]. The ratio between Pd(II) and Pd(0) was 1.9/1 (Figure 3.1.17). The amounts of Ca, Mg, P and N were practically identical to those detected untreated $(Pd\text{-}EPS)_{aerob}$.

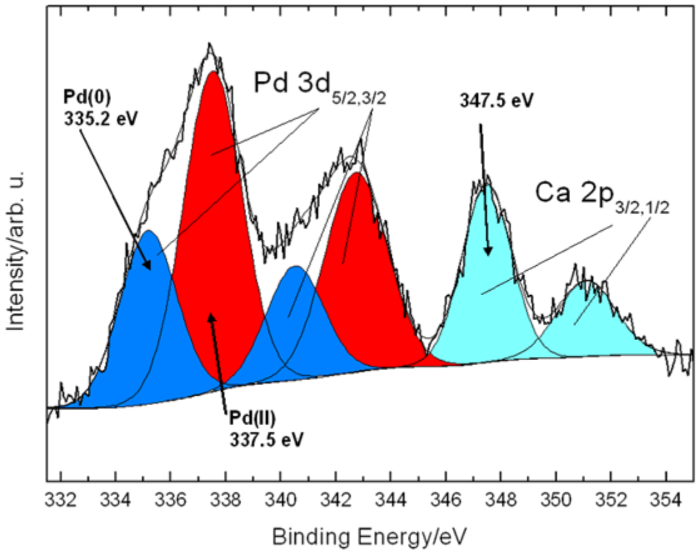


Figure 3.1.17. XPS spectrum of activated 1.7 % $(Pd\text{-}EPS)_{aerob}$

Normally Pd(II) is easily reduced to Pd(0) in the presence of H_2 in an aqueous system as well as in bacterial cultures[58] but in this case only about 30% of Pd (II) was reduced. This unexpected and surprising result can be tentatively explained. Excluding the presence of sulfur ligands, as demonstrated by the XPS analysis, the partial reduction could be ascribed to the fact that a relevant amount of palladium ions should not be bound to the –COOH and-OH groups of the polysaccharide but it should be present as different palladium species, more difficult to reduce at the present reaction conditions. The initial hypothesis was that a palladium phosphate could be formed. As matter of fact the production of some metal phosphates during the growth of a parent species of *Klebsiella oxytoca* such as *Citrobacter sp.*, another microorganism able to ferment citrate, was reported in the literature[59,60]. IR spectrum of Pd-EPS was so registered (Fig. 3.1.18B), evidencing a strong peak at 1068 cm^{-1} that is quite typical of the asymmetric stretching of a phosphate ion[61,62]. Moreover, the relevant presence of phosphorous, determined by XPS measurements, could further support the hypothesis of palladium phosphates formation in the isolated material. However, preliminary X-ray data would seem to exclude any Pd-P bond, evidencing, on the contrary, the formation of inorganic phosphate salts, such as $MgNH_4PO_3$. An alternative hypothesis could be the formation of palladium oxides covered by a layer of ionic Pd(II) species bonded to the polysaccharide functional groups and included in the sugar matrix, in a way similar to that observed when Fe-EPS was prepared. This PdO could be so more resistant to the reduction to Pd(0) in applied activation conditions[49].

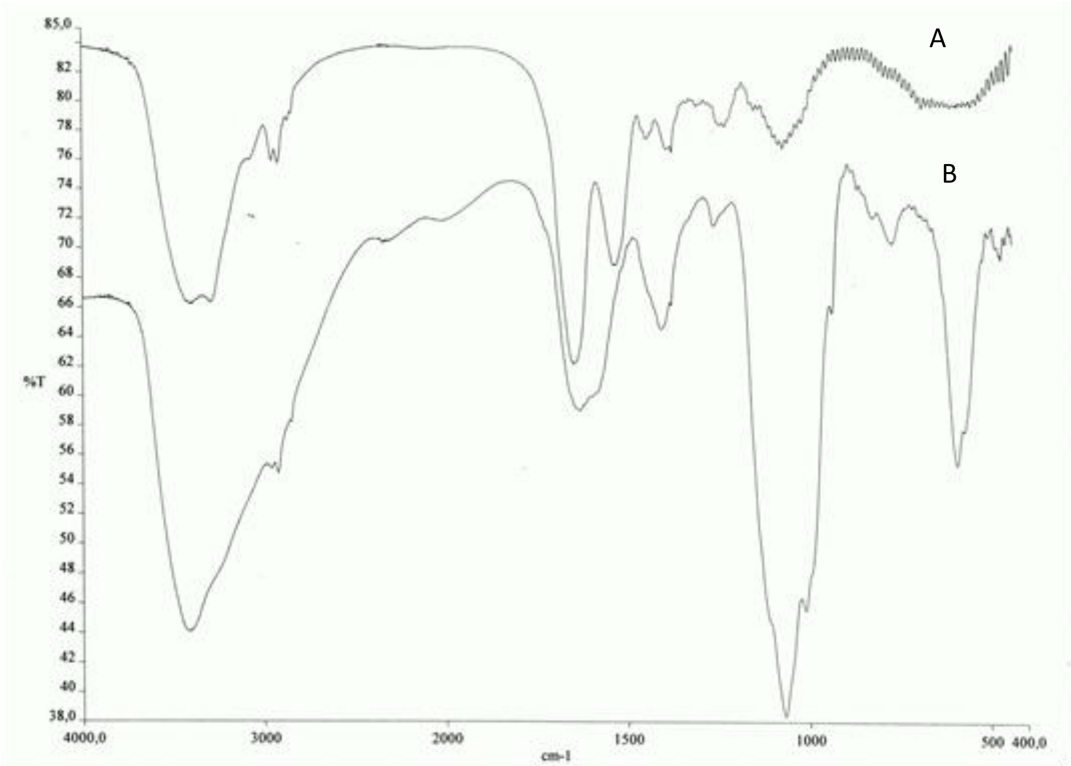


Figure 3.1.18. (A) IR spectrum (KBr pellets) of Na-EPS (metal-free-EPS); (B) IR spectrum (KBr pellets) of 1.7 % (Pd-EPS)$_{aerob}$.

For comparison a Pd-EPS was prepared also by an exchange reaction between iron-free-EPS (Figure 3.1.18 A) and an acetone solution of palladium acetate. Also these Pd-species showed the same IR profile of biogenerated Pd-EPS but the behavior of the former species was very different: in particular, explorative hydrodechlorination experiments of chlorobenzene showed that (Pd-EPS)$_{aerob}$, directly produced during the microorganism growth, was much more active affording benzene in 45% yield after 22 h at 30°C and 0.1 MPa of H_2, while the palladium species prepared by the exchange reaction produced benzene in only 6% yield[54,63]. These results highlight the relevance of the preparative method that may affect the availability and stability of the metal bound to the polysaccharide in water. Based on these results it was decided to use only Pd-EPS directly produced during the fermentation process of *Klebsiella*.

Another important observed fact was that Pd-EPS species are able to catalyze the reaction only when they are suspended in water. When the activity test was carried out only in organic solvent, no reaction was observed. This behavior can be explained by hypothesizing that water blowing the polymer structure allows to palladium to be available for the catalysis; in particular, it was supposed that starting from a twisted exopolysaccharide with the metal atoms embedded in it, water can unroll this structure so rendering palladium less hindered and more available to act as catalyst[54].

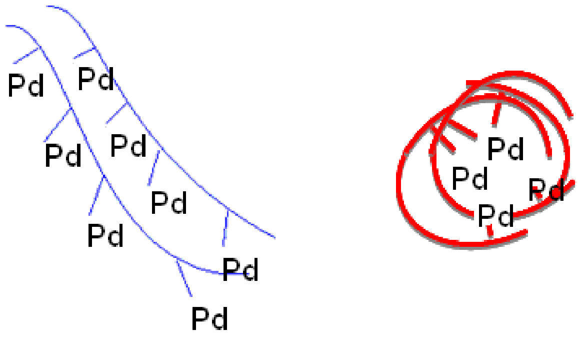


Figure 3.1.19. The exopolysaccharide could assume a spatial arrangement that does not hinder the interaction between metal and reagents when the catalyst is suspended in water (left), while it assumes a compact conformation in the presence of organic solvent (right)

For this reason, 1.7 % $(Pd\text{-}EPS)_{aerob}$ and other obtained biogenerated species were used in aqueous dispersion.

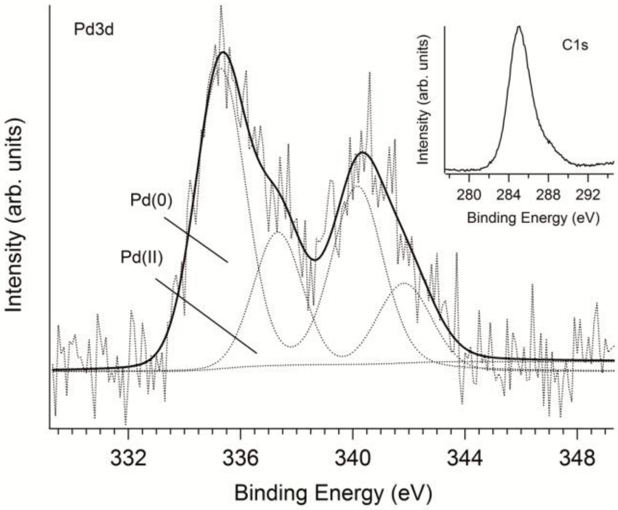
3.1.2.2 Preparation and characterization of 13 % $(Pd\text{-}EPS)_{anaerob}$[64]

In order to increase the production of Pd-EPS species and the amount of immobilized metal it was decided to try to change the fermentation conditions of *Klebsiella oxytoca*, working under anaerobic static mode. In this way $(Pd\text{-}EPS)_{anaerob}$ was obtained and the content of metal was 13%.

Citrate is transported by suitable up-take systems and it is catabolized throughout tricarboxylic acid cycle with oxygen, whereas without oxygen it is fermented to acetic acid and CO_2[48,65].

The citrate respiration is a faster process, so permitting to produce 1.7 % $(Pd\text{-}EPS)_{aerob}$ catalyst in a shorter time (3 days). However $(Pd\text{-}EPS)_{anaerob}$, obtained under anaerobic conditions, has a higher amount of palladium (13 %) determined by atomic absorption and was obtained in higher yield, in fact after 7 days 300 mg/L (based on dry weight) were recovered. Furthermore, it is important to note that, in this case, the amount of recovered Pd from colture is 78 % versus only 7 % recovered when the production was performed under aerobic conditions.

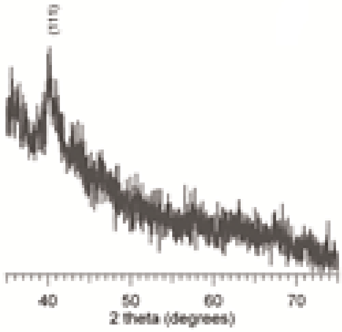
Also $(Pd\text{-}EPS)_{anaerob}$ was analyzed with different techniques to determine differences with the species obtained by aerobic fermentation. The high resolution XPS spectrum in Pd3d region is reported in figure 3.1.20. Along with the j = 5/2 and 3/2 splitting components, a significant broad band-shape profile is observed suggesting the presence of palladium species characterized by different oxidation states and/or chemical environments. Spectral deconvolution of the Pd3d line resulted into two doublet components. The main $3d_{5/2}$ spin-orbit peaks are centered at BE = 335.4 eV and 337.5 eV and are assigned to Pd(0) and Pd(II) species, respectively[51,56]. By comparing the relative intensities of Pd(0) and Pd(II) components, it was determined that Pd(0) accounts for *ca.* 70% of the total metal content in the sample. The C1s line (Fig 3.1.20, see inset) showed a single peak centered 285.0 eV with a shoulder on the higher BE energy side. The band-shape is consistent with the presence of a polysaccharide environment, where C-C, C-O, and C=O carbon components are expected. The main contribution to the core line at 285.0 eV is typical for hydrocarbon species ($-CH_n-$) arising from the alkyl moieties of the polysaccharide skeleton, whereas the components at BEs ranging between 286.5 and 290 eV can be ascribed to the polysaccharide groups containing oxidized carbon atoms[56].



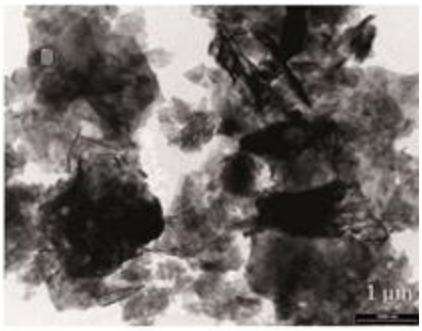
3.1.20. XPS spectrum of 13 %$(Pd\text{-}EPS)_{anaerob}$

More interesting is the XRD analysis of this sample. The corresponding diffraction pattern (Figure 3.1.21) revealed the presence of three peaks centred at 2θ≈40.1° (111), 46.5° (200) and 68.1°

(220), characteristic of crystalline *fcc* Pd. The mean crystallite size, as evaluated by the Scherrer equation, is *ca.* 5 nm. Further signals were not observed in the spectrum, suggesting the absence of any other detectable crystalline phase related to oxidized palladium species such as PdO, which has its major diffraction peak at 2θ≈34° (101)[66,67]. Accordingly, the microscopic structure of the catalyst prepared during the fermentation process resulted very different. The micrographs of 13% $(Pd\text{-}EPS)_{anaerob}$ (Figure 3.1.22) showed sub-nanoparticles, which roll over each other with an avalanche effect due to the polysaccharide matrix in between.



3.1.21. XRD analysis of 13 %$(Pd\text{-}EPS)_{anaerob}$



3.1.22. Micrographs of 13 %$(Pd\text{-}EPS)_{anaerob}$

3.1.2.3 Preparation and characterization of bimetallic EPS species

The field of heterogeneous catalysis, specifically catalysis on bimetallic alloys, has seen many advances over the past few decades. Bimetallic catalysts often show different and distinct electronic and chemical properties from their parent metals. This fact offers the opportunity to obtain new catalysts with enhanced selectivity, activity, and stability. Bimetallic catalysts started to gain considerable commercial interest in the 1960s for their use in hydrocarbon reforming, because they displayed activities unlike those of the monometallic catalysts[68]. The findings of these unexpected properties of bimetallic catalysts have inspired many extensive investigations on their possible applications. Currently bimetallic catalysts are widely utilized in many catalytic and electrocatalytic applications[68]. However, it is difficult to know *a priori* how the electronic and chemical properties of a particular bimetallic surface will be modified relative to the parent metals.

There are two factors that contribute to the modification of the electronic and chemical properties of a metal in a bimetallic surface:

1. the formation of heteroatom bonds changes the electronic environment of the metal surface, giving rise to modifications of its electronic structure through the ligand effect;
2. the geometry of the bimetallic structure is typically different from that of the parent metals, e.g., the average metal−metal bond lengths change, resulting in the strain effect that is known to modify the electronic structure of the metal through changes in orbital overlap.

The preparation of bimetallic catalysts is not simple. Metal segregation, sintering, surface enrichment of one of the metal and the inherent heterogeneity of bimetallic particles make the preparation of highly dispersed and uniform bimetallic particles difficult by using conventional impregnation or slurry methods technology. The dispersion of the active phase on the surface of the support is influenced by many factors such as the nature of support, method of preparation, calcination conditions, precursor and metal loading. In order to overcome these difficulties, many preparation techniques have been developed such as the use of organometallic precursors, reductive deposition-precipitation, electroless deposition, colloidal synthesis and many others[68].

Since *Klebsiella oxytoca* produces an exopolysaccharide with a well-defined and repeated structure, it was possible to think that the incorporation of two metallic species such as palladium and iron could follow a certain biological mechanism in order to obtain a potential bimetallic catalyst with defined structure. The presence of iron might increase the activity and selectivity of palladium, but, more important, being iron a nutrient for the cell, its presence may increase the production of Me-EPS.

During this thesis work, two different bimetallic species Pd-Fe-EPS were prepared and their characteristics are reported in Table 3.1.3.

Table 3.1.3. Amount of Fe and Pd and obtained yield of biogenerated Pd-Fe-EPS species

Catalysts	Pd (%)	Fe (%)	Yield (mg/L)	% of recovered Pd	% of recovered Fe
8.4 % Pd-Fe-EPS	8.4	7.4	450	76	67
2.2 % Pd-Fe-EPS	2.2	14	1100	48	55

Preliminary EXAFS analyses on both catalysts did not show any Pd-Fe bonds while P-O-Pd bridges were found. Furthermore, Pd nanoparticles were detected in both catalysts and from XPS analysis, high presence of Pd(0) was determined[69].

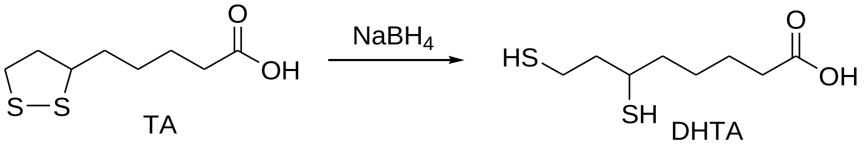
3.1.2.4 Preparation and characterization of Rh-EPS

At the light of the interesting results obtained by preparation of different Pd-EPS species, it was also decided to prepare other metal-EPS materials. In particular, a new Rh-EPS was obtained by fermentation of *Klebsiella oxytoca* under anaerobic conditions. As in the case of palladium, also rhodium is not a nutrient for the cell. In order to obtain EPS without iron, the fermentation was carried out by using Na-citrate; after 48 h, $RhCl_3$ was added and after further 72 h of incubation the Rh-EPS was obtained. 560 mg of catalyst with 1.2 % of Rhodium content, determined by atomic absorption, was recovered. The amount of recovered Rh from colture is 13 %.

3.1.3 Preparation and characterization of new water-soluble Rh-based catalysts

As reported in Chapter 1 water, as environmentally friendly solvent, implies the use of intrinsically soluble metal complexes such as hydroxides and aqueous-complex or the utilization of hydrophilic ligands.

During this thesis work and a contemporary research investigation in the group where this thesis was performed[70], it was decided to investigate the possibility to use a simple easily available and low cost molecule as ligand for rhodium species. The attention was focused on dihydrothioctic acid (DHTA). This compound has two thiolic groups in position 6 and 8 of the chain, potentially able to complex rhodium. In addition, the carboxylic group, once salified, could strongly promote the solubility of the complex in water and behave as further coordination site. Ideally, it would be possible to obtain a soluble complex in water, potentially suitable in aqueous biphasic catalysis. DHTA is easily obtained by reduction of thioctic acid (TA) with $NaBH_4$[71].



Scheme 3.1.1. Reduction of thioctic acid (TA) into dihydro thioctic acid (DHTA)

Thioctic acid, also known as α-lipoic acid, is the oxidized form of DHTA and is widely available on the market at low price. It is a natural molecule present in low concentration in the human body with essential functions in aerobic metabolism. It is produced industrially and sold as dietary supplement and antioxidant. DHTA is not very soluble in water, but after salification with Na_2CO_3, the salt form (DHTANa) is obtained. This form is soluble in water and for this reason, DHTANa can be used as bidentate ligand for rhodium species. In this way, it would be possible to obtain a catalytic species working in aqueous medium or in aqueous biphasic system.

The catalytic species was prepared by introducing the $[Rh(COD)Cl]_2$ (COD = 1,5-cyclooctadiene) in a deaerated aqueous solution containing the sodium salt of DHTA, by using a molar ratio ligand/metal 1:1. The solution obtained was stocked under nitrogen at 4°C and showed good stability for medium-long time, maintaining its catalytic activity.

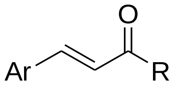
This obtained system was analyzed by ^1H-NMR and IR spectroscopy. Comparing the proton spectra of the free ligand with the spectra of the complex, a chemical shift of the signal attributed to the proton near to thiolic groups was observed. The proton on C_6 shifted from 2.92 to 3.61 ppm while the signals of the protons on C_8 shifted from 2.60 to 3.12 ppm. This would indicate a possible chelation of the sulfur atoms to the metal center. The infrared spectrum of the complex, recorded between 4400 and 400 cm^{-1}, showed the disappearance of the band at 2560 cm^{-1} due to SH stretching, present in the spectrum of the free ligand[72].

3.2 Synthesis of 4-(2-methoxynaphthalen-6-yl)butan-2-one (Nabumetone) (II) by hydrogenation of (E)-4-(2-methoxynaphthalen-6-yl)but-3-en-2-one (I)

Nabumetone (**II**) is an anti-inflammatory pharmaceutical active ingredient (API) belonging to the family of non-steroidal anti-inflammatory drugs (NSAIDs) and is used in the symptomatic treatment of rheumatic and inflammatory conditions[73].

Nabumetone (**II**) is a saturated ketone and can be prepared by selective hydrogenation of the corresponding α-β unsaturated ketone, (E)-4-(2-methoxynaphthalen-6-yl)but-3-en-2-one (**I**)[35]. The research of catalysts for the hydrogenation of multifunctional compounds such as an α-β unsaturated carbonyl substrate also containing an aromatic or heteroaromatic rings is still of great interest. The aim is to prepare very active and regioselective catalysts able to operate under mild conditions and to minimize the formation of by-products such as saturated alcohol, unsaturated alcohol and products of partial or total hydrogenation of aromatic or heteroaromatic rings.

In the literature the reduction of this type of C=C double bond in a generic structure as reported in Figure 3.2.1 is described.



Ar = aryl; R = H, alkyl, phenyl

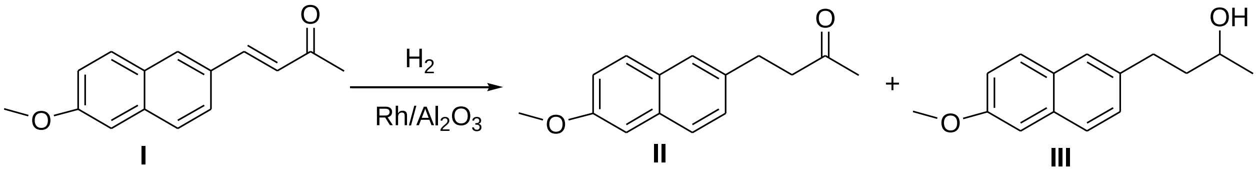
Figure 3.2.1. General structure of and α-β-unsaturated substrate

For example, the hydrogenation was carried out in methanol at room temperature and low pressure using a non-commercial 1 % Rh/$AlPO_3$ with a substrate/catalyst weight ratio between 300 and 500. The preparation of this catalyst is not simple and it was also reported that the reaction is fast although it is sensitive to the steric hindrance[74]. 5 % Rh on charcoal or alumina operating with a substrate/catalyst weight ratio equal to 20 or 10 % Pd on charcoal (in the presence of pyridine as co-catalyst), operating with a substrate/catalyst weight ratio equal to 100, are the preferred catalysts for the hydrogenation of hindered carbonyl compounds[75,76]. Recently, the efficient use of Cu/SiO_2 catalyst in the selective hydrogenation of **I** to obtain **II**, by using a substrate **I**/catalyst molar ratio 100/1 was reported[77]. It should be noted that the main advantage of this catalyst is the use of economics and safety copper. This last feature, as already reported, may be important especially when the catalysts are used in drugs synthesis.

Thus, in order to avoid the presence of metallic contaminants in the final hydrogenated product and in order to be able to reuse the catalytic system several times, it is necessary to select a suitable support that does not release the metal.

During this thesis work, the reduction of the compound **I** was investigated by using the home made catalyst 0.18 % Rh/Al_2O_3. Indeed, a 0.15% Rh/Al_2O_3 catalyst had already shown a very good activity in the above hydrogenation process on industrial scale[35]. In this case the used rhodium catalyst had been used up to 20 times (with small additions of fresh catalyst) but it was never recovered from the reaction mixture being always maintained inside the reactor. The goal of this work was to evaluate the activity of our catalyst in a bench reaction on lab. scale, in particular in recycling experiments after separation and recovery of the catalyst from the reaction mixture. Furthermore, since the characterization of the catalyst had not been reported, both fresh and recovered catalysts after a hydrogenation reaction were characterized by CO chemisorption analysis as reported below at paragraph 3.2.2.

3.2.1 Results and discussion



Scheme 3.2.1 Selective hydrogenation of C=C double bond of I catalyzed by 0.18% Rh/Al_2O_3

Table 3.2.1 Reduction of C=C double bond of I

Run[a]	P (MPa)	T (°C)	t (h)	Conv I (%)	II (%)	III (%)	TOF
1	0.5	50	1	99	99	---	3714
2	0.5	30	1,5	97	97	---	1016
3[b]	0.2	30	20	64	64	---	119
4[c]	0.5	50	20	92	92	---	370
5[d]	0.5	50	1	93	93	---	4656
6[d]	Recycling			80	80	---	4000

[a] reaction conditions: substrate **I** = 0.147 g (0.00065 mol); Rh/Al_2O_3 0.18 % = 0.010 g (substrate **I**/catalyst molar ratio 3700/1); toluene = 5 mL; [b]same conditions of run 1; [c] substrate **I** = 0.200 g (0.00088 mol); Rh/Al_2O_3 0.18 % = 0.006 g (substrate **I**/catalyst molar ratio 8000/1); toluene = 5 mL; [d] substrate **I** = 3.56 g (0.016 mol); Rh/Al_2O_3 0.18 % = 0.180 g (substrate **I**/catalyst molar ratio 5000/1); toluene = 20 mL.

Carrying out the reaction under milder conditions, high activity and selectivity towards C=C double bond were observed (run 1 and 5, Table 3.2.1) and maintained unchanged also when the catalyst was recycled (run 6, Table 3.2.1). However, it is also important to remark that better selectivity of catalyst was obtained when the reaction was performed with a high substrate **I**/catalyst molar ratio. In fact, in an experiment, not reported in Table 3.2.1, carried out at 50°C and 0.5 MPa of H_2 pressure and using a substrate **I**/catalyst molar ratio equal to 1000/1, a quantitative conversion of substrate **I** was observed but 2 % of alcohol **III** and 5 % of the sum of two other by-products were also detected.

3.2.2 Characterization of the catalyst

The catalyst used in run 5 (Table 3.2.1) was analyzed by CO chemisorption, after pre-treatment with hydrogen, by adopting the procedure reported in the experimental part(paragraph 5.13.7):

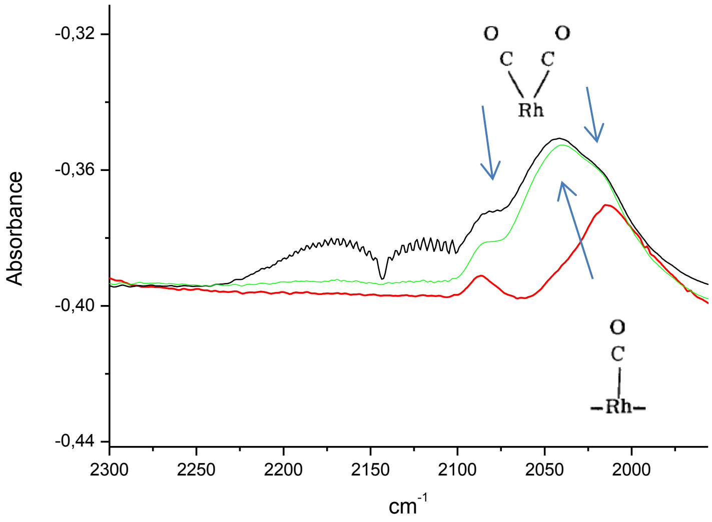


Figure 3.2.2. IR spectrum of used Rh/Al_2O_3 0.18 % catalyst in the Rh-carbonyls signal area
Legend: black line: CO sending; green line: first expansion of CO; red line: expansion with vacuum pump connected

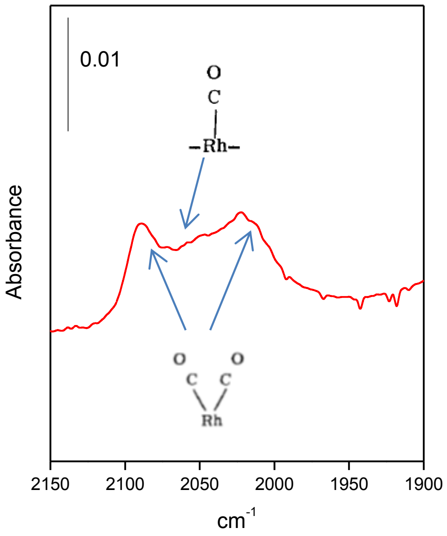


Figure 3.2.3. CO chemisorption analysis of fresh 0.18 % Rh/Al_2O_3

Compared to the fresh catalyst (Figure 3.2.3, already reported and discussed in Chapter 3.1), it is possible to observe the very intensive signal due to Rh-CO monocarbonyl species at 2050 cm^{-1}, which is so strong to partially cover the signals due to the geminal Rh-CO_2 bands absorption (2086 cm^{-1} and 2015 cm^{-1}). This is probably due to the partial aggregation of Rh particles. In order to verify the potential structural changes due to pre-treatment with hydrogen, the un-treated catalyst was analyzed but no interesting data were obtained probably due to the oxidation of rhodium.

3.2.3 Conclusions

The home made 0.18 % Rh/Al_2O_3 is very active toward the hydrogenation of C=C double bonds working under mild conditions. The procedure of patent[35] was confirmed but more detailed information was obtained. A partial agglomeration of Rh particles was determined by CO chemisorption analysis and this could explain the slight loss of activity. Furthermore, the presence of organic residues on the analyzed catalyst, despite a washing treatment, was detected and this might have influenced also this reduced activity (Figure 3.2.4). The used catalyst was analyzed to determine the metal content and obtained result confirm no leaching phenomena occoured.

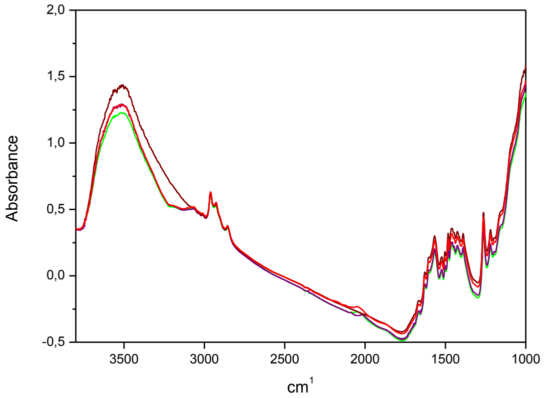
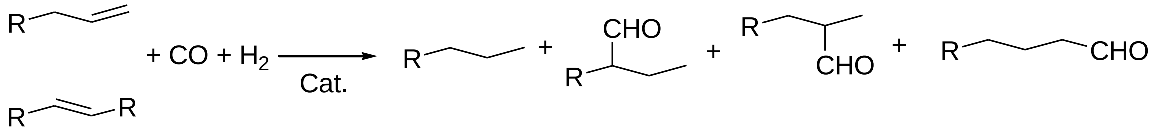


Figure 3.2.4. IR spectra of catalysts from 3500 to 1000 cm^{-1} after first use. The signal of organic residue is present at 1000 cm^{-1}

3.3 Hydroformylation of styrene (IV): introduction

The reaction of hydroformylation, also known as oxo-synthesis, is a catalytic reaction which allows to obtain aldehydes from an olefin by addition of CO and H_2 to the carbon-carbon double bond[19]. This process is widely used in industry to synthesize many oxo-products, important intermediates for a large number of compounds ranging from pharmacologically active compounds, agrochemicals and perfumes. The study and choice of the catalytic system is fundamental to increase the yield toward the desired product. One problem regards the chemo-selectivity of the reaction; in fact the hydroformylation catalysts may induce hydrogenation and isomerization side reaction. As far as the regio-selectivity of the reaction is concerned, the problem is the attack of the formyl group –CHO on the carbon atoms of the C=C double bond, so affording a mixture of aldehydes (Scheme 3.3.1)[19].



Scheme 3.3.1. Possible products obtainable in the hydroformylation reaction

It is known that main hydroformylation catalysts are cobalt-, rhodium- and platinum-based complexes, usually containing phosphino ligands. While platinum was profitably used only in the lab-scale, cobalt and rhodium were also applied in industry and the most efficient catalysts in terms of both activity and selectivity are rhodium complexes, capable to operate under mild conditions[11,19].

Hydroformylation is one of the most important industrial reaction catalyzed by soluble metal complexes. The major drawback of this homogeneous process is the separation of the expensive catalyst from the product mixture that requires an energy intensive process such as distillation. For this reason and for the consideration of the environmental aspects of the chemical production, liquid-liquid biphasic systems have been developed in these last years, with the catalyst confined in one of the two phases and the product in the other phase[18,78]. In particular, the use of environmentally more benign solvents such as water has been developed and the hydroformylation process represents one of the most striking example of this catalytic methodology. Since 1984, C_4 and C_5 aldehydes from propene or butene are produced by using the aqueous biphasic OXEA process (former Ruhrchemie/Rhône-Poulenc) catalysed by the water soluble complex Rh/TPPTS (TPPTS = triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt)[79,80]. Besides phosphines as TPPTS, many other new ligands and/or surfactants having different hydrophilic groups such as –COOH, NR_3, -OH, etc. have been designed to prepare new water soluble catalytic precursors[18]. It is known that also natural compounds, such as aminoacids, peptides, proteins and sugars are able to bind metallic species and maintain them soluble and active in water [81,82].

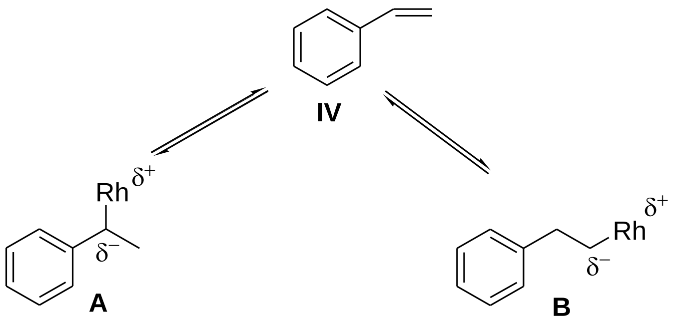
However, the aqueous biphasic process presents some drawbacks as the low solubility in water of high molecular weight olefins[83], the possible degradation of the catalyst that can lead to inactive hydroxylated rhodium species in the hydroformylation reaction[84] or to the formation of inactive clusters with phosphine bridges[85]. To reduce these problems, some immobilized homogeneous catalysts have been developed by using organic support functionalized with diphenylphospine, tertiary amine, thiol groups or inorganic support[86].

Furthermore, the encapsulation of homogeneous catalysts in inorganic supports such as zeolites was also studied[87]. Finally, biopolymers as supports for transition metal catalysts have also attracted the interest of researchers due to their abundance in nature, renewability, biodegradability and non-toxicity[81].

Only few examples of hydroformylation reactions carried out by using heterogeneous rhodium based catalysts as Rh on activated carbon[88] or Rh on silica[89] have been reported but heterogeneous catalysts are not currently used industrially.

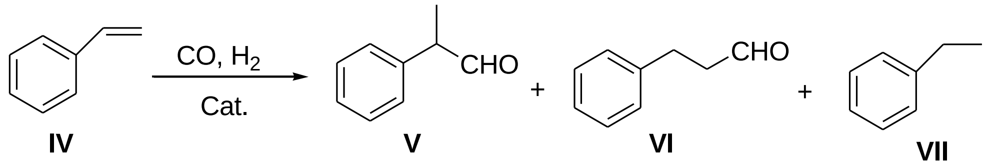
3.3.1 Discussions and results

To test the activity of Rh based home-made prepared catalysts (0.18 % Rh/Al_2O_3, 1.2 % Rh-EPS and RhDHTANa, see chapter 3.1.1.2, 3.1.2.4, 3.1.3 respectively) in the hydroformylation reaction, styrene (**IV**) was chosen as model substrate. A peculiarity of styrene hydroformylation is that the branched aldehyde is predominantly formed. This result has been explained by assuming a greater stability of the branched δ-alkyl intermediate (**A**) than to the linear isomer (**B**) formed in the catalytic cycle, because of the delocalization of the negative charge on the aromatic ring (Scheme 3.3.2)[19].



Scheme 3.3.2. The delocalization of the partial negative charge on the aromatic ring favors the formation of the branched aldehyde

The obtained results in the hydroformylation of **IV** are reported in Table 3.3.1, 3.3.2 and 3.3.3 by using the three different Rh-based species prepared during this work of thesis:



Scheme 3.3.3. Hydroformylation of IV

Table 3.3.1. Hydroformylation of IV catalyzed by 1.2 % Rh-EPS catalyst

Run	Conv. IV (%)	V (%)	VI (%)	VII (%)	V/VI	TOF
1	91	85	6	---	14	32
1° Recycle	79	74	5	---	15	27
2° Recycle	99	90	9	---	10	34
3° Recycle	88	83	5	---	17	31
4° Recycle	97	90	6	1	15	34

Reaction conditions: substrate **IV** = 0.025 g (0.24 mmol), 1.2 % Rh-EPS = 0.003 g (substrate **IV**/catalyst molar ratio 700/1); toluene = 2 mL; H_2O = 2 mL; p(H_2)=(CO)=3 MPa, T = 60°C; t = 20 h

The biogenerated catalyst 1.2 %Rh-EPS was used in the aqueous biphasic system and demonstrated a good activity (run 1, Table 3.3.1) in the hydroformylation of **IV** at 60°C and 6 MPa of syngas for 20 h (CO/H_2=1), by using a substrate **IV** to rhodium molar ratio 700/1. After any reaction, the aqueous catalytic phase was separated from the organic products and recycled four times (Table 3.3.1): in all recycles the catalyst showed high activity (79-99 %) and also high selectivity toward the branched aldehyde **V**.

Table 3.3.2 Hydroformylation of IV catalyzed by [Rh(DHTANa)]

Run	Conv. IV (%)	V (%)	VI (%)	VII (%)	V/VI	TOF
1	99	83	16	---	5	50
1° Recycle	97	82	15	---	5.5	50
2° Recycle	99	84	15	---	5.6	50
3° Recycle	98	80	18	---	4.4	49

Reaction conditions: substrate **IV** = 0.52 g (0.005 mol); toluene = 2 mL; 0.005 M H_2O solution of [Rh(DHTANa)]= 1 mL (substrate **IV**/catalyst molar ratio 1000/1), $p(H_2)=p(CO)=4$ MPa, T=80°C.

The aqueous biphasic of **IV** hydroformylation was carried out also by using [Rh(DHTANa)]. This catalyst showed a very good catalytic activity affording an almost quantitative substrate **IV** conversion after 20h at 80°C and 4 MPa of syngas (CO/H_2 = 1) (run 1, Table 3.2.3). The main product **V**, was obtained with 85 % selectivity. The aqueous catalytic phase was recycled three times and both activity and selectivity remained practically unchanged.

Table 3.3.3 Hydroformylation of IV catalyzed by 0.18 % Rh/Al_2O_3

Run	Conv. IV (%)	V (%)	VI (%)	VII (%)	V/VI	TOF
1	99	93	6	---	15.5	40
1° Recycle	99	92	7	---	13	40
2° Recycle	99	90	9	---	10	40
3° Recycle	50	44	2	4	22	20

Reaction conditions: substrate **IV**= 0.364 g (0.0035 mol); Rh/Al_2O_3 0.18 % = 0,8 g (substrate **IV**/catalyst molar ratio 800/1); toluene = 10 mL; $p(H_2)=(CO)=2$ MPa, T = 50°C; t = 20 h;

Also heterogeneous catalyst 0.18 % Rh/Al_2O_3 showed high activity and selectivity (run 1, Table 3.3.3) and after the use, it was recovered from the crude mixture by filtration on sintered glass filter. The catalyst was recycled three times and in the first two recycles the activity and selectivity remained high (1° and 2° recycling, Table 3.3.1) but in the third recycling the activity decreased. However, the selectivity towards the branched aldehyde **V** remained high and even increased. It should be noted that both the fresh catalyst and the recycled one were treated, before use, with 2 MPa of CO for 20 h at room temperature: as a matter of fact, when the reaction was carried out by using a not pre-activated catalyst, the formation of acetophenone was observed, probably due to oxygen absorption on catalyst surface.

3.3.2 Analysis of the recovered 0.18 % Rh/Al_2O_3 catalyst from the hydroformylation of styrene (IV)

The recovered catalysts after first use (run 1, Table 3.3.3) and third recycle (Table 3.3.3) were analyzed by FT-IR after CO chemisorption following the procedure described at paragraph 5.13.7.

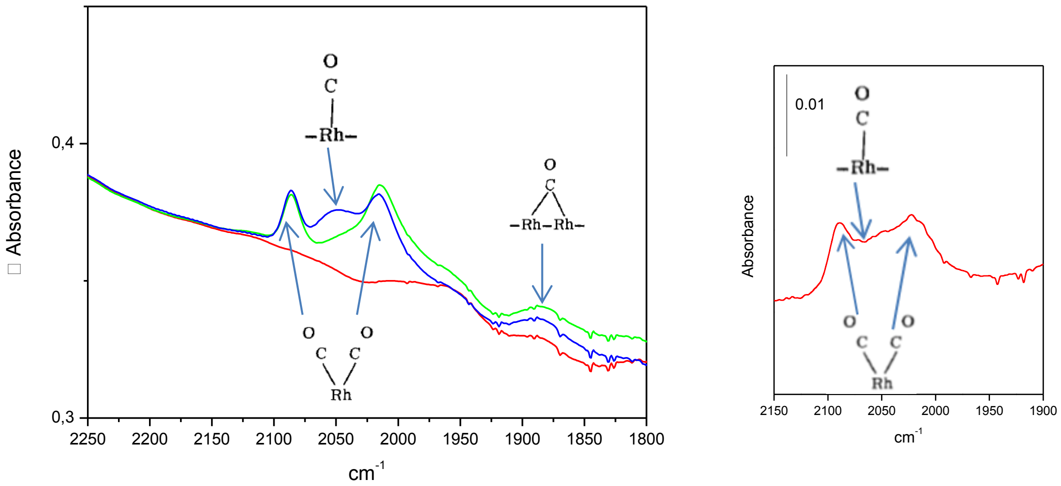


Figure 3.3.1. Left: analysis of 0.18 % Rh/Al_2O_3 catalyst after first use in styrene hydroformylation (run 1, Table 3.3.3) Legend: red line: background, blue line: first expansion of CO, green line: second expansion of CO (in vacuum); **Right: analysis of fresh catalyst**

From the obtained data, it is possible to observe in the used catalyst after first use (Figure 3.3.1 left) the formation of Rh_2-CO species at 1850 cm^{-1}, Rh-CO at 2050 cm^{-1} and Rh-$(CO)_2$ at 2086 cm^{-1} and 2015 cm^{-1}. The formation of Rh_2-CO and Rh-CO species is reversible and during CO expansion the signals attributed to these species decreased. On the contrary, the signal of $Rh(CO)_2$ remains essential constant because this species is formed in an irreversible way. The small increase of Rh-CO and Rh_2-CO observed in the recovered species is probably due to the increase in the size of rhodium particles: this enhancement of the rhodium particle size is also confirmed by TEM analysis:

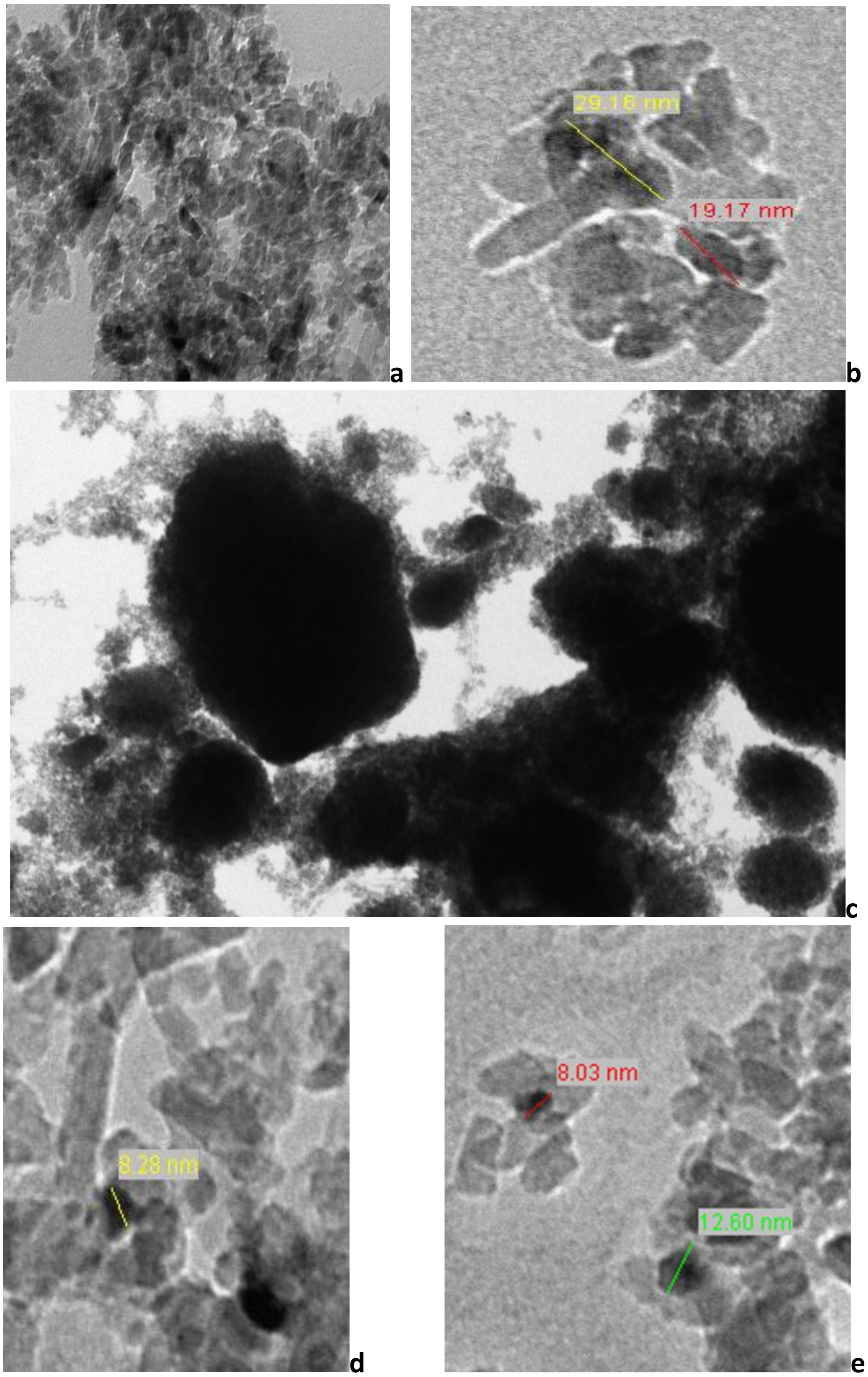


Figure 3.3.2. TEM analysis: a) and c): presence of deposition of organic material on the catalyst; b) particle size of rhodium after first use in the hydroformylation reaction; d) and e) particle size of rhodium in the fresh catalyst

As reported in Figure 3.3.2 it is possible to observe that after the first use in the hydroformylation reaction the size of rhodium particles increased with respect to the fresh catalyst. Furthermore, it was also observed deposition of organic material on the metal particles, also confirmed by IR analysis (Figure 3.3.3).

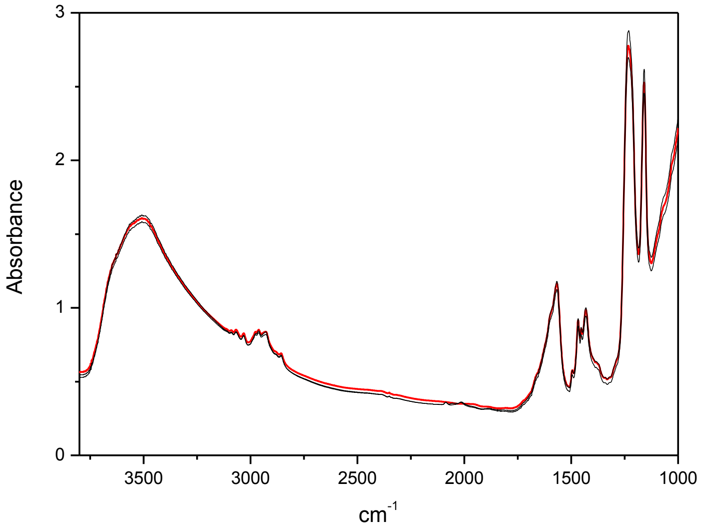


Figure 3.3.3. Strong absorption band at 1250 cm^{-1} is ascribable at deposition of organic material on catalyst.

Before CO chemisorption, the sample of used catalyst was analyzed after H_2 pre-treatment or without any pre-treatment. In this case, it was possible to demonstrate that H_2 treatment does not affect the catalyst structure (Figure 3.3.4) even if it is useful to clean the metal surface.

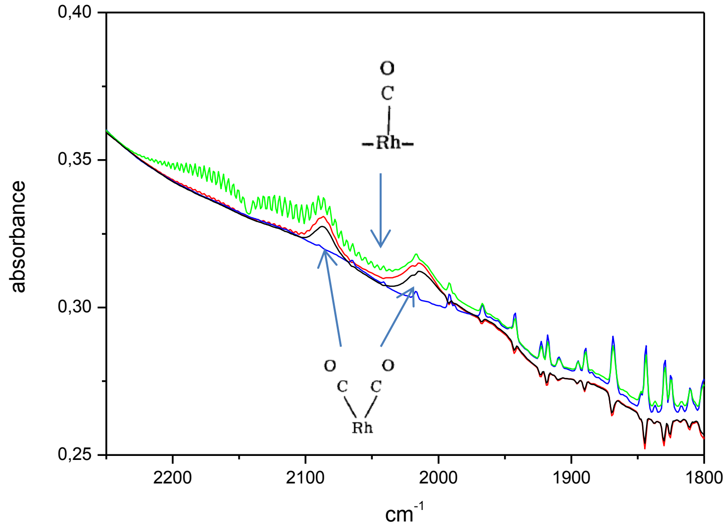


Figure 3.3.4. Analysis of recovered 0.18 % Rh/Al_2O_3 without H_2 treatment. Legend: blue line: background, green line: CO pulse, red line: first expansion, black line: second expansion

The catalyst recovered after the third recycle was also analyzed by CO chemisorption with pre-treatment with H_2:

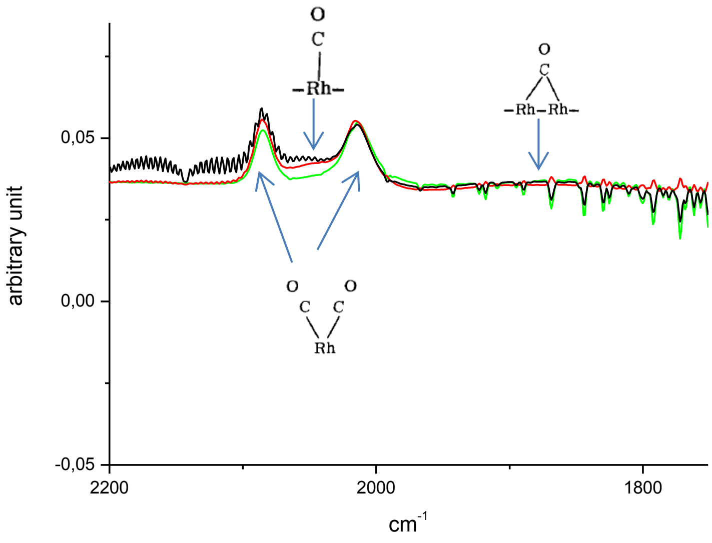


Figure 3.3.5. Obtained spectra from analysis of 0.18 % Rh/Al_2O_3 recovered from third recycle by chemisorption of CO. Legend: black line: CO pulse; red line: first expansion, green line: second expansion

Also in this case, it is possible to observe the formation of three rhodium species which confirm a partial aggregation of the metal particles could explain the reduction of activity. Furthermore, the possible organic residue due to not sufficient washing or due to partial polymerization of styrene (**IV**) could contribute to observe partial deactivation. Also in this case the recovered catalyst after the third recycle was analyzed and the content of Rh was not affected in relevant manner so excluding leaching phenomena.

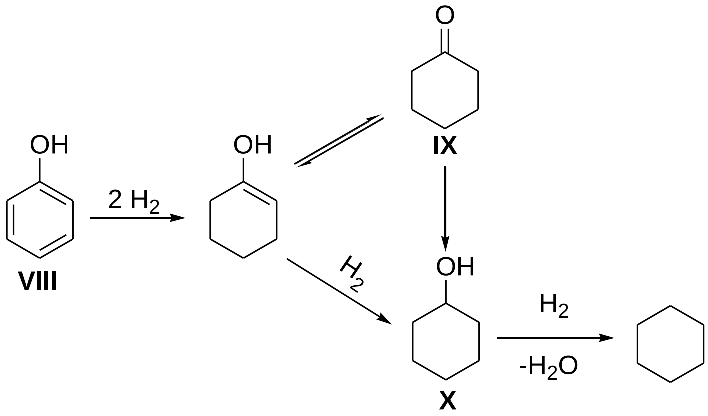
3.3.3 Conclusion

In conclusion, all the obtained catalysts were very active in the hydroformylation process of **IV** and they could be considered potentially interesting materials for industrial applications. In particular, it is important to underline the performances of 0.18 % Rh/Al_2O_3, especially in the light of the fact that only few examples of heterogeneous catalysts are reported in the literature for this type of reaction. Slight differences in regioselectivity and activity on the three catalytic materials were observed, but no attempt to identify the best experimental conditions for each of them and to choose the best hydroformylation catalyst was made for the moment.

3.4. Hydrogenation of phenol (VIII): introduction

The hydrogenation of phenol (**VIII**) is an important reaction in the chemical industry. The most important product formed by this reaction is cyclohexanone (**IX**) which is a key intermediate for the production of caprolactam for nylon 6 and of adipic acid for nylon 6,6[90].

However this reaction is not selective and a mixture of **IX** and cyclohexanol (**X**) is usually obtained. For this reason, the most widely used industrial method to obtain **IX** is the cobalt catalyzed oxydation of **X** or of cyclohexane[90]. It is clear that a selective process that allows to obtain directly and selectively **IX** from **VIII** may represent a simpler synthetic way, which is energetically more efficient and may produce lower amount of wastes (Scheme 3.4.1)[91].



Scheme 3.4.1. Possible hydrogenation pathways of phenol (VIII)

The selective synthesis of **IX** by the one-step hydrogenation is largely dependent on the catalyst properties and up to now a widely range of catalysts have been designed for this reaction, mostly carried out in gas phase. However, liquid-phase hydrogenation of phenol (**VIII**) might increase the selectivity to cyclohexanone (**IX**) since the reaction could be performed at lower temperature[91].

Nowadays, the selective hydrogenation of phenol (**VIII**) is industrially carried out in gas phase at 0.1-0.2 MPa of H_2 in the presence of Pd/Al_2O_3 catalysts promoted with alkali or alkaline earth metals which improve activity, selectivity and stability of the catalyst[92].

More recently, many catalytic systems have been developed by using different supports[93] or Pd nanoparticles[94] or different metals such Rh and Ru[95,96] to improve the selectivity toward **IX**.

As concerning the support, the influence of base or acid sites has been deeply studied. Several reports indicate that base sites favor the formation of **IX** whereas other authors indicate that acid sites promote this process. The acid-base property seems to have influence on the adsorption-desorption equilibrium of reactants and products, being responsible of the selectivity towards the reaction products[93]. It is reported that **VIII** adsorbed in nonplanar way on the base sites of support should gives **IX**, on the contrary when it is adsorbed on the acid sites in the coplanar way the formation of **X** should be preferred[97]. However, probably the mechanism is more complex and the presence of acid sites could favor also the isomerization of intermediate cyclohex-1-enol into cyclohexanone (**IX**)[98].

As far as the hydrogenation of **VIII** is concerned, this reaction is also important from an environmental point of view. Compounds containing a phenol moiety are starting compounds and/or intermediates in many industrial processes and are present in effluents from coke ovens, oil refineries, petrochemical units and polymers production and agricultural wastes. These wastewaters are characterized by variable concentrations of phenolic compounds (500–4000 mg/L)[99]and the full reduction of these compounds can lead to the formation of less toxic products[100]. For example in Table 3.4.1 the toxicity of phenol (**VIII**) and their hydrogenation products (**IX** and **X**) are reported.

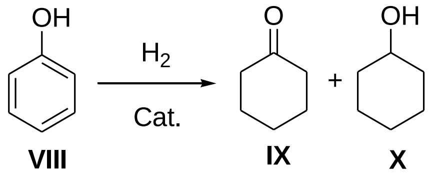
Table 3.4.1. Toxicity of compounds VIII, IX and X[100]

Compound	EC 50 (mg/L)*	T.U (1000 mg/L solution)**
Phenol (VIII)	15.9	63
Cyclohexanone (IX)	11.6	86
Cyclohexanol (X)	18.5	54

*EC 50: half maximale effective concentration; **T.U: toxicity unit

3.4.1.Results and discussion

The hydrogenation of **VIII** was carried out by using 0.28 % Pd/Al_2O_3 and 0.18 % Rh/Al_2O_3. When the reaction was performed in the presence of Pd catalyst with **VIII**/Pd molar ratio 1000/1, at 60°C and 0.5 MPa of H_2 in water, after 1 h, only 26 % conversion into **IX** was found. By using 0.18 % Rh/Al_2O_3 better results were obtained (Table 3.4.2).



Scheme 3.4.2. Reduction of VIII

Table 3.4.2. Reduction of VIII catalyzed by 0.18 % Rh/Al_2O_3 at different conditions

Run	T (°C)	t (h)	Conv.VIII (%)	IX (%)	X (%)
1[a]	50	20	99	69	30
2[a]	1° Recycle		84	71	13
3[a]	2° Recycle		18	15	3
4[a]	60	5	99	54	45
5[a]	60	1	88	76	12
6[b]	60	1	99	59	40

Reaction conditions: [a] substrate **VIII** = 0.016 g (0.17 mmol); 0.18 % Rh/Al_2O_3 = 0.010 g (substrate **VIII**/catalyst molar ratio 1000/1); H_2O = 10 mL; p(H_2) = 0.5 MPa; [b] same conditions of run 1 but 10 mL of cyclopentyl methylether was used.

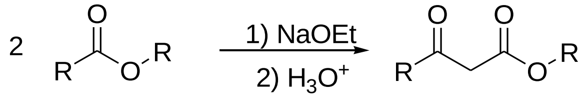
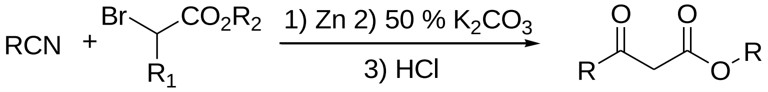
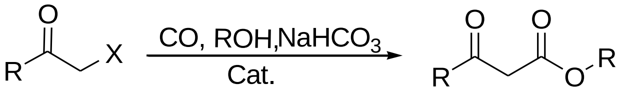
Good selectivity and activity were obtained when the reaction was carried out at 60°C for 1 h (run 5, Table 3.4.2).

Fine activity and selectivity towards the formation of **IX** were observed working in water with very high substrate **VIII**/Rh molar ratio, at lower temperature or reducing the reaction time (runs 1 and 5, Table 3.4.2); on the contrary, increasing reaction time or working in organic solvent (cyclopentyl methylether) the formation of **X** increases (run 4 and 6, Table 3.4.2). It was also possible recycle the catalyst at least for one re-use. It is to underline that working with minimum amount of catalyst (0.010 g), it was decided to recover and re-use directly the water phase after extraction with toluene without filtration or other cleaning treatment of the catalyst. In conclusion, the homemade 0.18 % Rh/Al_2O_3 results competitive respect to the performance reported for other Rh catalysts operating in different solvents and conditions (supercritical CO_2 or ionic liquid)[95,96]. Furthermore, unlike what reported in the literature for other rhodium based catalysts, this catalyst does not seem to cause the hydroxyl group elimination[101]. A deeper study to optimize the reaction parameters is forecast in the next future.

3.5 Synthesis of β-Ketoesters: introduction

β-Ketoesters are widely used in many different industrial fields. For example, pyridines and pyrazolones are prepared from reaction between β-ketoesters and nitrogen-containing binucleophiles[102,103,104,105]. Other uses regard the preparation of a few ligands for homogeneous catalysis[106], the production of sols and polymers using sol-gel technology[107], the synthesis of biologically active compounds such as agrochemicals[108,109].

Table 3.5.1. Some methods to obtain β-ketoesters

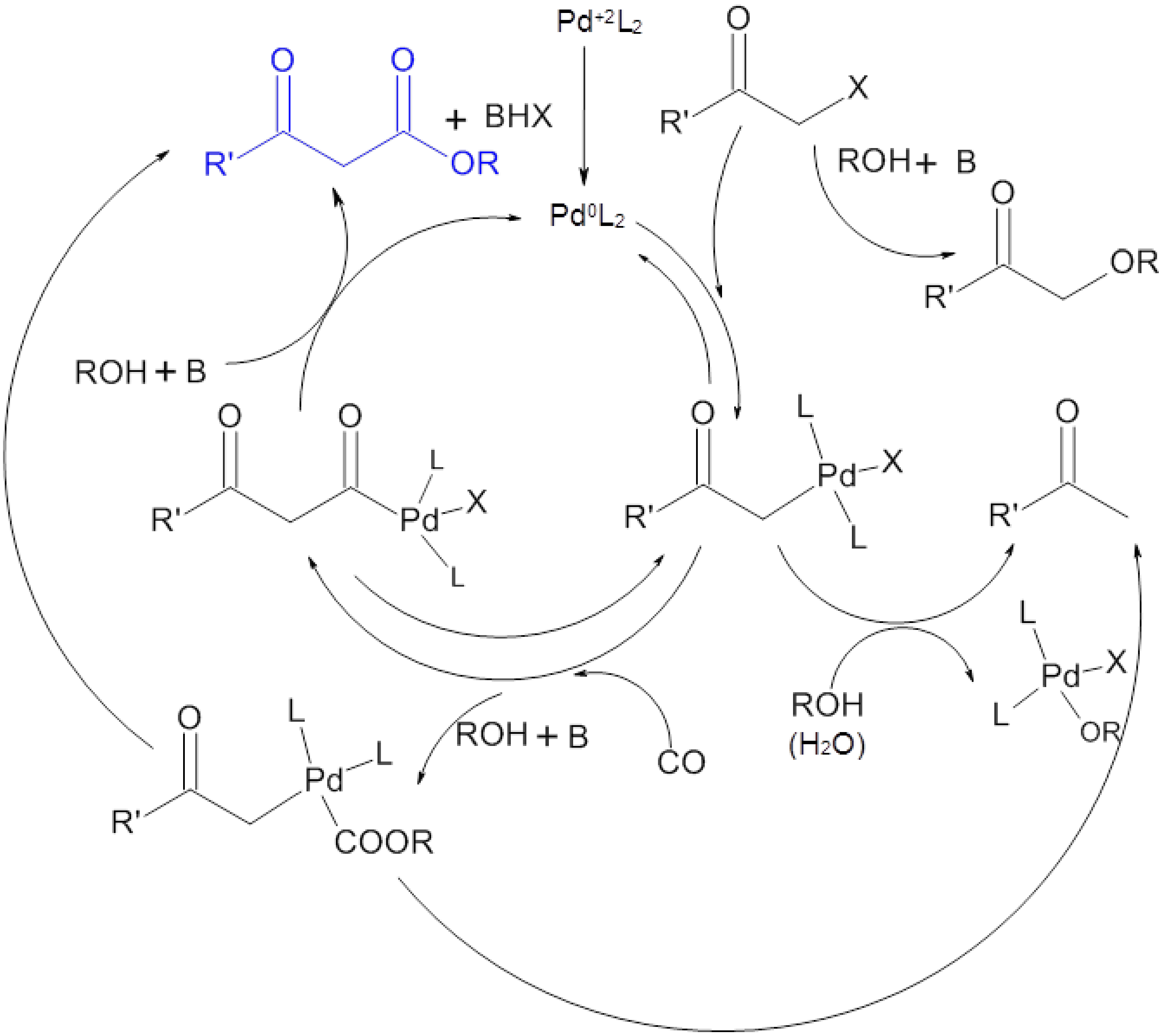
Claisen condensation

Blaise reaction

Carbonylation reaction


In order to prepare β-ketoesters in high yield a multitude of synthetic methods have been investigated[110], however, the classical used methods are Claisen condensation and Blaise reaction. In Claisen condensation, two esters are condensed in the presence of one or more equivalents of a strong base. In Blaise reaction, α-bromocarboxylic ester reacts with a nitrile in the presence of one or more equivalent of zinc. However these methods have several drawbacks. For example, Claisen and related condensation processes suffer from the need of a stoichiometric amount of an expensive strong base, while Blaise reaction suffers of use of expensive nitriles and generates a large waste stream because a stoichiometric amount of zinc and ammonium salt are produced as by-products[109].

The synthesis of β-ketoesters by carbonylation of α-haloketons appears a good choice even if toxic CO is required; in fact the reaction can be carried out in a one step and the desired product can be easily recovered from the crude mixture, and minor quantities of wastes are produced. However, data on the carbonylation of α-haloketones by using palladium catalysts are scanty, probably due to the low yields obtained if the reaction parameters are not fine tuned[111].

Usually, the carbonylation of α-haloketons is carried out by using CO pressure ranging between 0.3 and 10 MPa, in the presence of transition metal based catalysts, often using as solvent the same alcohol present in the final ester group. The optimal pressure depends on the structure of substrate and in general the yield increases when the pressure of CO increases. Good results on acyclic substrates were obtained with pressure values between 1.5 and 4 MPa. To neutralize the acid formed during the reaction, either organic bases, such as tertiary amines, or inorganic bases have been used, usually in excess with respect to the substrate. The reaction is often carried out by using Pd-based homogeneous catalysts in the presence of phosphines. These kinds of catalysts have shown high activity also at milder conditions, compared to rhodium or cobalt based catalysts[112,113]. Before research activities of the group in which this thesis was made[114], at the best of our knowledge, heterogeneous Pd-catalyst have been claimed in reference[108] for the synthesis of β-ketoesters by carbonylation of haloketones. Indeed, the activity of heterogeneous catalysts could be due to the formation of colloidal palladium species with a behavior similar to homogeneous catalyst; at the end of the reaction, these colloidal species could be absorbed again on the support but the recovered material could be different in structure and in activity[115].

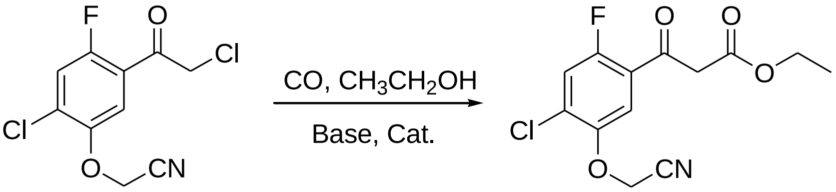
The possible mechanism of reaction under homogeneous conditions is reported in the following Scheme 3.5.1.



Scheme 3.5.1. Possible catalytic cycle for the carbonylation of α-haloketones by using Pd-based homogeneous catalysts

The first step of the reaction is the oxidative addition of the α-haloketone to Pd atom. Subsequently there is the addition and insertion of CO followed by the reductive elimination of the β-ketoester. During the reaction some by-products can be formed. For example alcohol or another potential nucleophile can react with the reactant by nucleophilic substitution. Other side reactions can form ketone derivatives. To synthesize a high amount of β-Ketoester is necessary to use a good catalyst that minimizes the undesired reactions, to check the reaction parameters and to dose the reagents correctly.

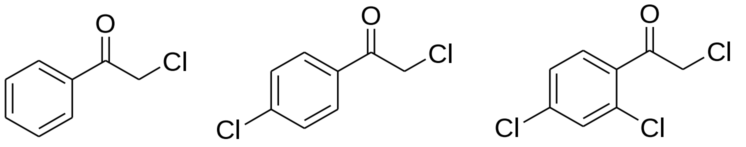
In the literature the carboxymethylation of α-chloroketones to obtain β-ketoesters as intermediate for agrochemicals is reported[108,109] and for example, the synthesis of ethyl 3-(4-chloro-5-(cyanomethoxy)-2-fluorophenyl)-3-oxopropanoate is described in reference[108].



Scheme 3.5.2. Synthesis of ethyl 3-(4-chloro-5-(cyanomethoxy)-2-fluorophenyl)-3-oxopropanoate by carbonylation

In this patent[108] the synthesis of a family of chloroketones is claimed by carboxymethylation of α-chloroketones in the presence of Pd catalysts and phosphorous ligand. In particular it reports good activity and selectivity by using homogeneous and heterogeneous catalysts in different conditions; reactions are carried out in inert solvents or in the same alcohol used as nucleophile, too. Subsequently, a Japanese patent[116] claimed the use of peculiar phosphorous ligands to obtain good results.

Preliminary carbonylation tests were carried out on three different of α-chloroketones having a structure similar to that of the industrial compound precursor of ethyl 3-(4-chloro-5-(cyanomethoxy)-2-fluorophenyl)-3-oxopropanoate (Figure 3.5.1):



XI **XII** **XIII**

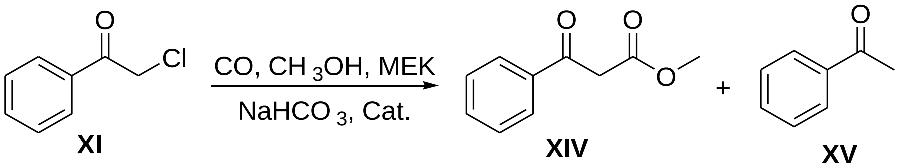
Figure 3.5.1. Selected substrates

The carboxymethylation of these substrates have already been investigated in the laboratory where this thesis was made, by using homogenous and heterogeneous Pd-based catalysts in the presence of phosphine under different conditions (p(CO)=1.5-5 MPa, T=80-100°C) but, besides the formation of the desired compounds, the hydrogenolysis of C-Cl was detected especially when the substate **XIII** was studied[117].

In order to improve the selectivity of this reaction, during this thesis work, the new homemade heterogeneous catalysts 0.28 % Pd/Al_2O_3 and 13 % $(Pd\text{-}EPS)_{anaerob}$ were tested. Furthermore, for comparison, the reaction was investigated by using a 5 % Pd/C commercialized by Chimet s.p.a. This catalyst was chosen because it is very active in various types of industrial processes. It is also important to note that the all reactions were performed in absence of phosphine in order to make the process more economical.

3.5.1 Results and discussion

In Table 3.5.2, encouraging results, obtained by using 2-chloro-1-phenylethanone (**XI**) as starting material, are reported:



Scheme 3.5.3. Carboxymethylation of 2-chloro-1-phenylethanone (XI)

Table 3.5.2. Carboxymethylation of 2-chloro-1-phenylethanone (XI) in the presence of different Pd catalysts

Run	Catalyst	t (h)	p(CO) MPa	Conv. XI (%)	XIV (%)	XV (%)	Other by-products (%)	TOF
1[a]	Pd/Al_2O_3 0.28 %	22	4.5	84	84	---	---	19
2[a]	Pd/Al_2O_3 0.28 %	22	2.5	81	73	8	---	18
3[a]	Pd/Al_2O_3 0.28 %	5	2.5	37	37	---	---	37
4[b]	Pd/C 5 %	5	2.5	36	30	6	---	36
5[a]	Pd/Al_2O_3 0.28 %	2	2.5	35	35	---	---	87.5
6[b]	Pd/C 5 %	2	2.5	34	34	---	---	85
7[c]	$Pd(OAc)_2/PPh_3$	18	2.5	99	89	10	---	5.5
8[c]	$Pd(OAc)_2/PPh_3$	4	4	99	93	6	---	18

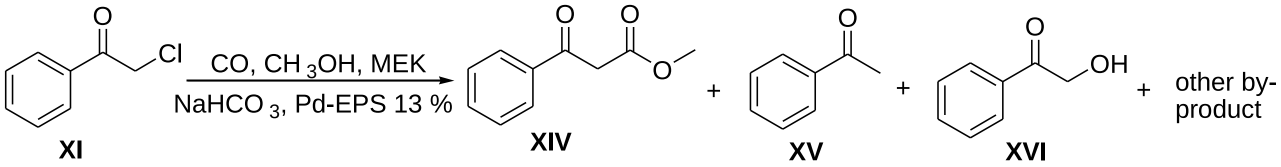
Reaction conditions: [a] substrate **XI** = 0.154 g (0.001 mol); CH_3OH = 0.256 g (CH_3OH/**XI** molar ratio 8/1); Pd/Al_2O_3 0.28 % = 0.076 g (substrate **XI**/catalyst molar ratio 500/1); $NaHCO_3$ = 0.126 g (base/substrate **XI** molar ratio 1.5/1); methylethylketone (MEK) = 7 mL; T = 100°C, [b] 0.008 g of Pd/C 5 % (substrate **XI**/catalyst molar ratio 500/1, the catalyst is 50 % wet); [c] 0.0022 g of $Pd(OAc)_2$ (substrate **XI**/catalyst molar ratio 100/1) and 0.005 g of PPh_3 (PPh_3/Pd molar ratio 2/1) was used as catalyst. Before use, the catalyst was dissolved into 5 mL of MEK and activate at p(CO) = 1 MPa for 2 h.

The reactions were performed by using $NaHCO_3$ as base and with a controlled amount of CH_3OH (alcohol/**XI** molar ratio 8/1). The used catalysts were the homemade 0.28 % Pd/Al_2O_3 and the commercially available 5 % Pd/C (Chimet), in absence of phosphine as metal-stabilizer.

The amount of CH_3OH is important for the reaction selectivity, in fact higher amount of alcohol can lead to the formation of byproducts by nucleophilic substitution but a too low amount can reduce the reaction rate. Low reaction rates were observed also carrying out the reaction in the presence of low amount of solvent MEK.

Before performing the reaction, all the catalytic systems were activated under 1 MPa of CO for 2 h at 25°C. For comparison the reaction was also carried out by using the homogeneous catalyst $Pd(OAc)_2$ in the presence of triphenylphosphine (runs 7 and 8, Table 3.5.2). It was found that the selectivity of the reaction is better when the homemade 0.28 % Pd/Al_2O_3 was used rather than the homogeneous catalyst (run 1 compare to run 7 of Table 3.5.2). Furthemore, it also possible to observe that the activities of 0.28 % Pd/Al_2O_3, 5 % Pd/C and of the homogenous system are absolutely comparable. It is important to remark that in the presence of $Pd(OAc)_2/PPh_3$ the substrate **XI**/catalyst molar ratio was 100/1 while when the heterogeneous catalyst was used this ratio was 500/1. Noteworthy, by comparing the homemade catalyst (0.28 % Pd/Al_2O_3) with 5 % Pd/C, the metal content of the first catalyst is 1/20 of the latter one and therefore 0.28 % Pd/Al_2O_3 is definitely cheaper.

The synthesis of methyl 3-oxo-3-phenylpropanoate (**XIV**) was also carried out in an aqueous biphasic system by using the biogenerated catalyst 13 % $(Pd\text{-}EPS)_{anaerob}$.



Scheme 3.5.4. Synthesis of XIV by using 13 % $(Pd\text{-}EPS)_{anaerob}$ as catalyst

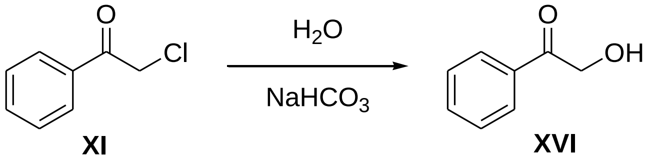
The obtained results are reported in the Table 3.5.3.

Table 3.5.3. Carboxymethylation XI in the presence of 13 % Pd-EPS

Run	Conv. XI (%)	XIV (%)	XV (%)	XVI (%)	Other by-products (%)	TOF
1[a]	99	51	5	19	24	25
2[b]	99	23	8	29	39	25
3[c]	76	28	3	5	40	19

Reaction conditions: [a] substrate **XI** = 0.3 g (0.0020 mol); CH_3OH = 0.49 g (CH_3OH/substrate **XI** molar ratio 8/1, d=0.79 g/mL); 13 % $(Pd\text{-}EPS)_{anaerob}$ = 0.0032 g (substrate **XI**/catalyst ratio 500/1); $NaHCO_3$ = 0.245 g (base/substrate **XI** molar ratio 1.5); MEK = 7 mL; H_2O = 2 mL; p(CO) = 4.5 MPa; T = 100°C; t = 20 h; before use, the catalyst was active dispersed into 2 mL of H_2O and activated at p(CO) = 1 MPa for 2 h; [b] experiment carried out with catalyst recovered from run 1; [c] substrate **XI** = 0.3 g (0.0020 mol); CH_3OH = 0.49 g (CH_3OH/substrate **XI** molar ratio 8/1, d=0.79 g/mL); 13 % $(Pd\text{-}EPS)_{anaerob}$ = 0.0032 g (substrate **XI**/catalyst ratio 500/1); $NaHCO_3$ = 0.245 g (base/**XI** molar ratio 1.5); MEK = 7 mL; H_2O = 0.06 mL; p(CO) = 4.5 MPa; T = 100°C; t = 20 h; the catalyst was not activated before use.

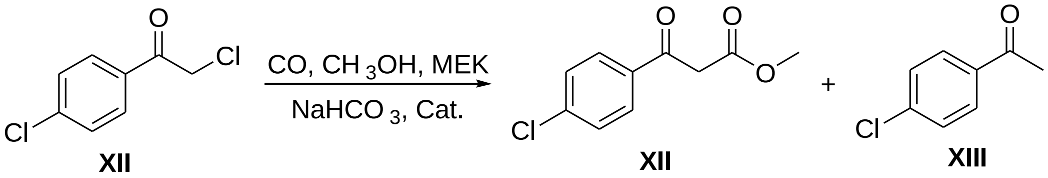
13 % $(Pd\text{-}EPS)_{anaerob}$ shows an excellent activity and good recyclability but a very poor selectivity, with formation of both acetophenone (**XV**) and 2-hydroxy-1-phenylethanone (**XVI**). While the formation of acetophenone (**XV**) as by-product is due to the mechanism above described, the formation of 2-hydroxy-1-phenylethanone (**XVI**) is probably due to the nucleophilic substitution of chlorine atom by water in the presence of a base ($NaHCO_3$). The presence of water is unavoidable as the catalyst is almost inactive in the presence of only organic solvents (See paragraph 3.1.2.1).



Scheme 3.5.5. Possible formation of by-product by nucleophilic substitution

In order to improve the selectivity, the amount of water was reduced (run 3, Table 3.5.3) but the selectivity was not improved; moreover, the catalyst activity was decreased probably due to an insufficient swelling of the polysaccharide polymer.

The reaction of carboxymethylation was extended to 2-chloro-1-(4-chlorophenyl)ethanone (**XII**) (Table 3.5.4) and 2-chloro-1-(2,4-dichlorophenyl)ethanone (**XIII**) (Table 3.5.5). These compounds were chosen as model substrates as the industrial precursor of the agrochemical ethyl 3-(4-chloro-5-(cyanomethoxy)-2-fluorophenyl)-3-oxopropanoate presents halogen atoms on the aromatic ring and in presence of palladium catalyst, this halogen atoms could be removed so leading to the formation of undesired by-products. In this context, it was verified if homemade catalyst 0.28% Pd/Al_2O_3 was able to catalyze the carboxymethylation reaction without provoking the dehalogenation of the aromatic ring.

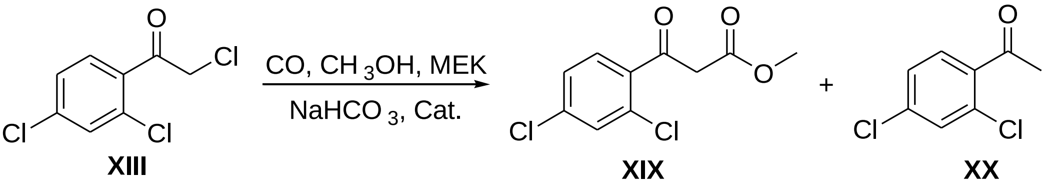


Scheme 3.5.6. Carboxymethylation of XII

Table 3.5.4. Carboxymethylation of 2-chloro-1-(2,4-dichlorophenyl)ethanone (XII) in the presence of different Pd catalysts

Run	Cat.	t (h)	p(CO) MPa	Conv. XII (%)	XVII (%)	XVIII (%)	Other by-products (%)	TOF
1[a]	Pd/Al_2O_3 0.28 %	22	4.5	99	93	6	---	23
2[a]	Pd/Al_2O_3 0.28 %	5	2.5	72	67	---	5	72
3[b]	Pd/C 5 %	5	2.5	71	46	---	25	71
4[a]	Pd/$Al_2O_3$0.28 %	2	2.5	42	36	---	6	53
5[b]	Pd/C 5 %	2	2.5	41	31	---	10	5
6[c]	Pd(OAc)$_2$/PPh$_3$	2	2.5	99	61	38	---	124
7[c,d]	Pd(OAc)$_2$/PPh$_3$	17	2.5	99	91	8	---	15

Reaction conditions: [a] substrate **XII** = 0.188 g (0.001 mol); CH_3OH = 0.256 g (CH_3OH/substrate **XII** molar ratio 8/1); Pd/Al_2O_3 0.28 % = 0.076 g (substrate **XII**/catalyst molar ratio 500/1); $NaHCO_3$ = 0.126 g (base/ substrate **XII** molar ratio 1.5/1); MEK = 7 mL; T = 100°C; [b] 0.008 g of Pd/C 5 % (substrate **XII**/catalyst molar ratio 500/1, the catalyst is 50 % wet) was used; [c] 0.0022 g of Pd(OAc)$_2$ (substrate **XII**/catalyst molar ratio 100/1) and 0.005 g of PPh$_3$ (PPh$_3$/catalyst molar ratio 2/1) were used; [d] T = 70°C.



Scheme 3.5.7. Carboxymethylation of 2-chloro-1-(2,4-dichlorophenyl)ethanone (XIII)

Table 3.5.5. Results obtained by carboxymethylation of 2-chloro-1-(2,4-dichlorophenyl)ethanone (XIII)

Run	Cat.	t (h)	p(CO) MPa	Conv. XIII (%)	XIX (%)	XX (%)	Other by-products (%)	TOF
1[a]	Pd/$Al_2O_3$0.28 %	4	2.5	87	87	---	---	54
2[a,b]	Pd/$Al_2O_3$0.28 %	4	2.5	75	75	---	---	47
3[a,b]	Pd/$Al_2O_3$0.28 %	4	2.5	70	70	---	---	44
4[a,b]	Pd/$Al_2O_3$0.28 %	4	2.5	66	66	---	---	41
5[c]	Pd/C 5 %	4	2.5	72	57	8	7	45
6[d]	Pd/$Al_2O_3$0.28 %	2	2.5	41	41	---	---	51
7[c]	Pd/C 5 %	2	2.5	39	38	---	1	49

Reaction conditions [a] substrate **XIII** = 0.444 g (0.002 mol); CH_3OH = 0.512 g (CH_3OH/substrate **XIII** molar ratio 8/1); Pd/Al_2O_3 0.28 % = 0.152 g (substrate **XIII**/catalyst molar ratio 500/1); $NaHCO_3$ = 0.252 g (base/substrate **XIII** molar ratio 1.5/1), MEK = 10 mL; T = 100°C; [a, b] the reaction was carried out by using the catalyst recovered from the previous run; [c] 0.008 g of Pd/C 5 % (substrate **XIII**/catalyst molar ratio 500/1, catalyst is 50 % wet) was used; [d] substrate **XIII** = 0,222 g (0.001 mol); CH_3OH = 0.256 g (CH_3OH/substrate **XIII** molar ratio 8/1); Pd/Al_2O_3 0.28 % = 0.076 g (substrate **XIII**/catalyst molar ratio 500/1); $NaHCO_3$ = 0.126 g (base/substrate **XIII** molar ratio 1.5/1), MEK = 5 mL, T = 100°C.

In both cases the homemade catalyst 0.28 % Pd/Al_2O_3 showed a good activity and selectivity toward the desired ketoesters (**XVII** and **XIX**). A further interesting feature was found when the reaction was carried out with the recycled catalyst (runs 2-4, Table 3.5.5): in fact, a slight decrease of activity was observed but the selectivity was maintained practically unchanged.

In conclusion during the present research work it was possible to identify a good-excellent homemade heterogeneous catalyst suitable for this kind of reaction. Scaling up of this reaction, as usual for any heterogeneous catalyzed reaction, could require a fine tuning of the reaction conditions but it seems promising for the production of a large variety of interesting industrial products and surely competitive with other known methods to produce β-ketoesters[110,111,112,113].

3.6 Hydrodechlorination of chlorinated organic compounds catalyzed by Pd-based catalysts: introduction

Chlorinated organic compounds are very common in the chemical industry. They can be used as intermediates or final products with different uses (solvents, pesticides, electrical insulators and heat transfer agents).

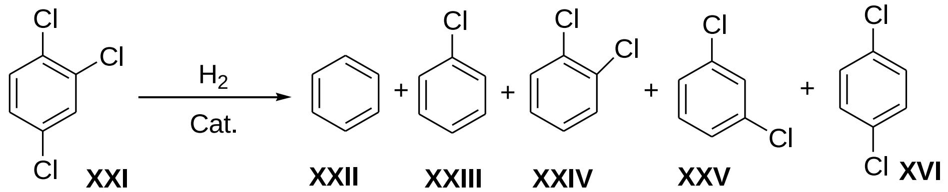
Due to their extensive use and their hydrophobic nature and high stability they are abundant in wastewaters, soils, sewage sludges, surface waters, groundwaters etc.[118]. Furthermore, they are toxic for human health and for environment in general and this feature has stimulated researches to find methods for reducing their concentration in the ecosystem.

Hydrodechlorination using Pd-based catalysts appears a good way to reduce the amount of these contaminants in water. In the literature it is reported that the hydrodechlorination may be highly selective and efficient in clean waters even at mild conditions[118]. However, palladium is very sensitive to deactivation, especially in waste-waters, due to its sequestration and precipitation with suspended organic matter. In addition, loss of Pd(0) reactivity is mostly due to hydrogen sulfide (H_2S and HS^-), which is ubiquitous in anoxic ecosystem[119,120]. Despite these difficulties, the interest for hydrodechlorination of wastewaters still remains high[118,121,122,123]. For these reasons, it was useful to verify the possibility of using homemade Pd-based catalysts in the hydrodechlorination of 1,2,4 trichlorobenzene (TCB, **XXI**) chosen as model substrate.

3.6.1 Hydrodechlorination by using heterogeneous catalysts

The hydrodechlorination of polychlorinated compounds by using Pd-based heterogeneous catalysts is widely described in the literature and it is reported that the decay of activity is due to the poisoning of the active phase by HCl formed in the reaction, by particle sintering and coke deposition, especially when the reaction is carried out in gas-phase[124]. Good results were obtained by using Pd/C, Pd/Al_2O_3 or bimetallic catalysts[125], working in water or in a biphasic system[126].

At the light of the literature data, it was decided to try the homemade catalyst 0.28 % Pd/Al_2O_3 under various conditions. In Table 3.6.1 the results obtained by using 0.28 % Pd/Al_2O_3 with a substrate/catalyst molar ratio 500/1 are reported:



Scheme 3.6.1. Hydrodechlorination of XXI catalyzed by 0.28% Pd/Al_2O_3

Table 3.6.1. Results obtained by using 0.28% Pd/Al_2O_3

Run	$p(H_2)$ MPa	T (°C)	Base	Conv. XXI (%)	XXII (%)	XXIII (%)	XXIV (%)	XXV (%)	XVI (%)
1[a]	3	80	---	10	---	5	5	Traces	Traces
2[b]	3	80	Et_3N	3	---	Traces	3	Traces	Traces
3[c]	3	80	$NaHCO_3$	12	---	6	6	---	---

Reaction conditions: All reactions was performed by using 0.28 % Pd/Al_2O_3 with substrate **XXI**/catalyst molar ratio 500/1 in 7 mL of H_2O; [a] substrate **XXI** = 0.024 g (0.13 mmol); 0.28 % Pd/Al_2O_3 = 0.010 g; t = 20 h; [b] same conditions of run 1 but 0.054 mL of Et_3N was added (Et_3N/substrate **XXI** molar ratio 3/1, d=0.726 g/mL); [c] same conditions of run 1 but 0.033 g of $NaHCO_3$ was added ($NaHCO_3$/substrate **XXI** molar ratio 3/1).

Preliminary results obtained by working at the above reaction conditions (Table 3.6.1) were not encouraging. At the light of these results, it was decided to reduce the substrate **XXI**/catalyst molar ratio to 100/1 and to vary other reaction parameters as temperature, solvent and base (Table 3.6.2).

Table 3.6.2. Results obtained by studying different reaction parameters

Run	p(H) MPa	T (°C)	Base	Solvent	Conv. XXI (%)	XXII (%)	XXIII (%)	XXIV (%)	XXV (%)	XXVI (%)
1[a]	3	80	$NaHCO_3$	H_2O	99	99	---	---	---	---
2[a]			Recycle		10	2	---	5	2	1
3[b]	3	80	---	H_2O	99	99	---	---	---	---
4[b]			Recycle		2	Traces		2	---	---
5[c]	3	80	Et_3N	H_2O	99	99	---	---	---	---
6[c]			Recycle		14	Traces	2	6	3	3
7[d]	3	30	---	H_2O	99	99	---	---	---	---
8[e]	0.2	30	$NaHCO_3$	H_2O	99	99	---	---	---	---
9[f]	0.2	30	$NaHCO_3$	MEK	30	2	4	18	4	2
10[g]	0.2	30	$NaHCO_3$	THF/H_2O	98	93	1	---	---	4
11[h]			Recycle		29	4	3	11	8	3
12[h]	0.2	30	$NaHCO_3$	H_2O	99	99	---	---	---	---
13[h]			Recycle		23	4	4	5	5	5
14[g]	0.2	30	TOA	H_2O	21	3	2	8	6	2

Reaction conditions: All reactions are performed by using 0.28 % Pd/Al_2O_3 with sub. **XXI** /catalyst molar ratio 100/1; [a] substrate **XXI** = 0.024 g (0.013 mmol); $NaHCO_3$ = 0.033 g ($NaHCO_3$/substrate **XXI** molar ratio 3/1); 0.28 % Pd/Al_2O_3 = 0.050 g; H_2O = 7 mL; t = 20 h; [b] same conditions of run 1; [c] same conditions of run 1 but 0.054 mL of Et_3N was added (Et_3N/substrate **XXI** molar ratio 3/1, d=0.726 g/mL); [d, e] same conditions of run 1; [f] same condition of run1 but 7 mL of MEK was used; [g] same conditions of run 1 but 7 mL of 1:1 mixture of THF/H_2O was used; [h] same conditions of run 1 but 0.066 g of $NaHCO_3$ ($NaHCO_3$/substrate **XXI** molar ratio 6/1) was used; T = 80°C; [g] same conditions of run 1 but 0.32 mL of trioctylamine (TOA) was used (TOA/substrate **XXI** molar ratio 6/1).

In many cases, working only in water under very mild conditions and in the presence or absence of base, complete dehalogenation of TCB **XXXIII** was observed but, despite the use of base, it has never been possible to recycle the catalyst efficiently. This fact could be due to the detachment of palladium from support, favored by the presence of water, and subsequent sintering of these colloidal palladium particles which then lose catalytic activity. It was also observed that the use of trioctylamine TOA brings significant disadvantages with respect to the use of triethylamine (run 14 versus run 5). To verify the possibility to recover and recycle the catalyst, other tests were carried out under different condition. Especially during the recycle tests, the prevailing formation of 1,2-dichlorobenzene **XXIV** among all the other dichoro isomers (**XXV** and **XXVI**) was observed. This result is quite predictable as the hydrodechlorination reaction rate is determined by steric and inductive effects. In particular, chlorine atoms next to each other show a lower reaction rate, while chlorine atoms adjacent to hydrogen atoms show a higher reaction rate[38].

Finally, hydrodechlorination was also investigated by using polymethyhydrosiloxane as H-donor. In these preliminary studies, 5 % Pd/C produced by Chimet s.p.a was used in absence of base and maintaining a substrate **XXI**/catalyst molar ratio 61/1:

Table 3.6.3. Hydrodechlorination of XXI catalyzed 5 %Pd/C (Chimet) under different reaction conditions

Run	T (°C)	Solvent	Conv. XXI (%)	XXII (%)	XXIII (%)	XXIV (%)	XXV (%)	XXVI (%)
1[a]	70	H_2O	24	3	1	14	3	3
2[b]	138	Xylene mix	15	1	---	8	3	3
3[c]	138	Neat	60	19	5	29	5	2

Reaction conditions: [a] substrate **XXI** = 0.1g (0.55 mmol); 5 % Pd/C = 0.038 g (substrate **XXI**/Catalyst molar ratio 61/1, 50 % wet); PMHS = 0.168 mL; H_2O = 10 mL; t = 20 h; [b] same conditions of run 1, but 10 mL of mixture of xylene was used; [c] substrate **XXI** = 2,11 g (2 mL); 5 % Pd/C = 1.15 g (substrate **XXI**/catalyst molar ratio 61/1, 50 % wet), PMHS = 2.5 mL, t = 20 h, neat conditions.

The reaction was carried out or in water (run 1, Table 3.6.3), or in a mixture of xylenes (run 2, Table 3.6.3) or under neat conditions (run 3, Table 3.6.3) but in all cases only partial dehydrodechlorination of 1,2,4-trichlorobenzene **XXI** was found. At the light obtained results, only the reaction under neat conditions deserves to be studied in more details.

3.6.2 Hydrodechlorination catalyzed by biogenerated activated 1.7 % $(Pd\text{-}EPS)_{aerob}$, 13 % $Pd\text{-}(EPS)_{anaerob}$, 8.4 % Pd-Fe-EPS and 2.2 %Pd-Fe-EPS

Reductive dehalogenation of chlorinated aromatic compounds by using different types of “bio-palladium” has already been reported. For example, Pd(0) precipitated sulphate-reducing SRB of *Desulfovibrio* genus was recently demonstrated. This biologically precipitated ‘‘bio-palladium’’ was a superior catalyst for dechlorination compared to Pd(0) prepared by chemical reduction under

H_2[59]. Its application for the treatment of contaminated soil and groundwater is well documented, including dechlorination of PCBs[52,127,128].

In this work of thesis, a possible alternative and innovative approach that combines the use of a microorganism and palladium sources in the presence of hydrogen or a hydrogen donor was proposed for hydrodechlorination by using new biogenerated Pd-EPS catalytic species.

For example, 1.7 % $(\text{Pd-EPS})_{\text{aerob}}$, worked very efficiently (up to 99% transformation) in relatively mild conditions by using a high molar ratio between the substrate and the precious metal (1000/1) in the hydrodechlorination of chlorobenzene[63]. However, the reactivity of polyhalogenated molecules in the reductive dehalogenation is usually lower than that of simple monohalogenated molecules and for this reason it was studied the activity of these catalysts toward different model molecules, such as 1,2,4-trichlorobenzene (**XXI**) in order to identify the most promising catalyst and the best reaction conditions.

Since the active catalytic species for the reductive dehalogenation is metallic palladium, activated 1.7 % $(\text{Pd-EPS})_{\text{aerob}}$, 13 % $(\text{Pd-EPS})_{\text{anaerob}}$ and the bimetallic species 8.4 % Pd-Fe-EPS and 2.2 % Pd-Fe-EPS were used due to their relevant content of Pd(0).

The obtained results by using these catalytic species are reported in the following Table 3.6.4.

Table 3.6.4. Results obtained by using different biogenerated species

Run	Catalyst	Molar ratio	Conv. XXI (%)	XXII (%)	XXIII (%)	XXIV (%)	XXV (%)	XXVI (%)
1[a]	1.7 % Act. Pd-EPS	61/1	5	---	---	3	1	1
2[b]	1.7 % Act. Pd-EPS	8/1	33	2	---	21	5	5
3[c]	13 % Pd-EPS	61/1	11	---	---	7	2	7
4[d]	13 % Pd-EPS	8/1	76	9	19	36	6	6
5[d,e]	13 % Pd-EPS	8/1	84	5	9	42	11	17
6[f]	2.2 % Fe-Pd-EPS	61/1	23	1	1	15	3	3
7[g,h]	2.2 % Fe-Pd-EPS	8/1	99	99	---	---	---	---
8[g,h]	2.2 % Fe-Pd-EPS	8/1	99	20	20	42	12	5
9[i]	8.4 % Fe-Pd-EPS	8/1	90	20	21	34	9	6
10[i,l]	8.4 % Fe-Pd-EPS	8/1	89	44	15	23	4	3

Reaction conditions: [a] substrate **XXI** = 0.356 g (0.00196 mol); Activated 1.7 % Pd-EPS = 0.201 g; H_2O = 2 mL; THF = 1 mL; $p(H_2)$=3 MPa; T = 60°C, t= 20 h; [b]same conditions of run 1 but 1.52 g of 1.7 % Pd-EPS was used; [c] substrate **XXI** = 0.356 g (0.00196 mmol); 13 % Pd-EPS = 0.026 g; H_2O = 2 mL; THF = 1 mL; $p(H_2)$=3 MPa; T = 60°C, t = 20 h; [d]same conditions of run 3 but 0.198 g of 13 % Pd-EPS was used; [e]experiment carried out by using the catalytic solution recovered from previous run and T = 80°C; [f] substrate **XXI** = 0.434 g(0.0024 mol); 2.2 % Pd-Fe-EPS: 0.190 g; $p(H_2)$= 3 MPa; H_2O = 2 mL; THF = 1 mL; T = 60°C; t = 20h; [h] same conditions of run 6 but 1.45 g of 2.2 % Pd-Fe-EPS was used, [h e]experiment carried out by using the catalytic solution recovered from previous run; [i] same conditions of run 6 but 0.38 g of 8.4 % Pd-Fe-EPS was used, [l] experiment carried out by using the catalytic solution recovered from previous run.

A first experiment, carried out in the presence of activated 1.7 % $(Pd\text{-}EPS)_{aerob}$ under the conditions reported in Table 3.6.4 gave to a very low conversion yield. A mixture of the three dichloro isomers (**XXIV**, **XXV** and **XXVI**) was produced: 1,2-dichlorobenzene (**XXIV**) represented the main product (run 1, Table 3.6.4), whereas chlorobenzene (**XXIII**) and benzene (**XXII**) were not formed at all. On the basis of previous experiments on chlorobenzene such a low activity was unexpected[63]. In order to improve the substrate conversion the experiment was carried out by adopting the same reaction conditions but with an increased catalyst amount (substrate **XXI**/Pd molar ratio = 8/1). In this case a 33% conversion was achieved and, as previously observed, again

XXIV was the main product (run 2, Table 3.6.4). Moreover, for the first time, even if in a very small amount, **XXII** was formed (2%) (run 2, Table 3.6.4).

Working in the presence of 13 % (Pd-EPS)$_{anaerob}$ under the condition reported in Table 3.6.5 with a substrate **XXI**/Pd molar ratio equal to 61/1 (run 3, Table 3.6.5) a modest (about 10%) activity was detected. Moreover, the three dichloro-isomers (**XXIV**, **XXV** and **XXVI**) were again the only reaction products (run 3, Table 3.6.4). On the other hand, by carrying out the reaction with a substrate (**XXI**)/Pd molar ratio = 8/1, about 76% of **XXI** was converted and, besides the three dichloro-derivatives (**XXIV**, **XXV** and **XXVI**), also 19% of **XXIII** and even 9% of **XXII** were found in the reaction mixture (run 4, Table 3.6.5).

Noteworthy, the aqueous catalytic phase was recovered and reused in a consecutive experiment by adding fresh **XXI**. Conversion was very high (84%), although a partial loss of the dehalogenating efficiency of the catalyst was observed. As a matter of fact, 13 % (Pd-EPS)$_{anaerob}$ afforded the three dichlorobenzene isomers (**XXIV**, **XXV**, **XXVI**) in relevant yield, even higher than in the first experiment, but further dehalogenation to **XXII** and **XXIII** was also observed.

The possible explanation of these results could be a structural change of the catalyst and/or a poisoning of the palladium surface. The metal leaching is excluded, as demonstrated by atomic absorption determination on the recovered catalyst.

Carrying out the reaction by using the bimetallic species 2.2 % FePd-EPS with a substrate (**XXI**)/catalyst molar ratio 61/1 (run 3, Table 3.6.4) and adopting the condition reported in Table 3.6.4, a low substrate **XXI** conversion was observed and 1,2 dichlorobenzene **XXIV** was the main product (15 %) while the other dichloro isomers (**XXV** and **XXV**) and both **XXIII** and **XXII** were formed in negligible amounts (run 3, Table 3.6.4). Reducing the substrate **XXI**/catalyst molar ratio up to 8/1 (run 7, Table 3.6.4) quantitative conversion into **XXII** was detected. Therefore, the aqueous catalytic phase was recovered and re-used in a consecutive experiment by adding fresh **XXI**. Again the conversion was complete but it was observed a partial loss of the dechlorination efficiency of the catalyst: now only 20% of **XXII** was formed and the main product was **XXIV** (Run 8, Table 3.6.4).

When 8.4 % Fe-Pd-EPS was used as catalyst, it showed a lower activity with respect to 2.2 % Fe-Pd-EPS and similar to that of the corresponding recycled 2.2 % Fe-Pd-EPS catalyst; it is however interesting the significant increase in the amount of **XXII** obtained by working at 80° C instead of 60°C (runs 9 and 10, Table 3.6.4). The catalytic activity of both Fe-Pd-EPS, especially that of fresh 2.2% Fe-Pd-EPS, was higher than that of 1.7 % (Pd-EPS)$_{aerob}$ and 13 % (Pd-EPS)$_{anaerob}$ (runs 2 and 4,

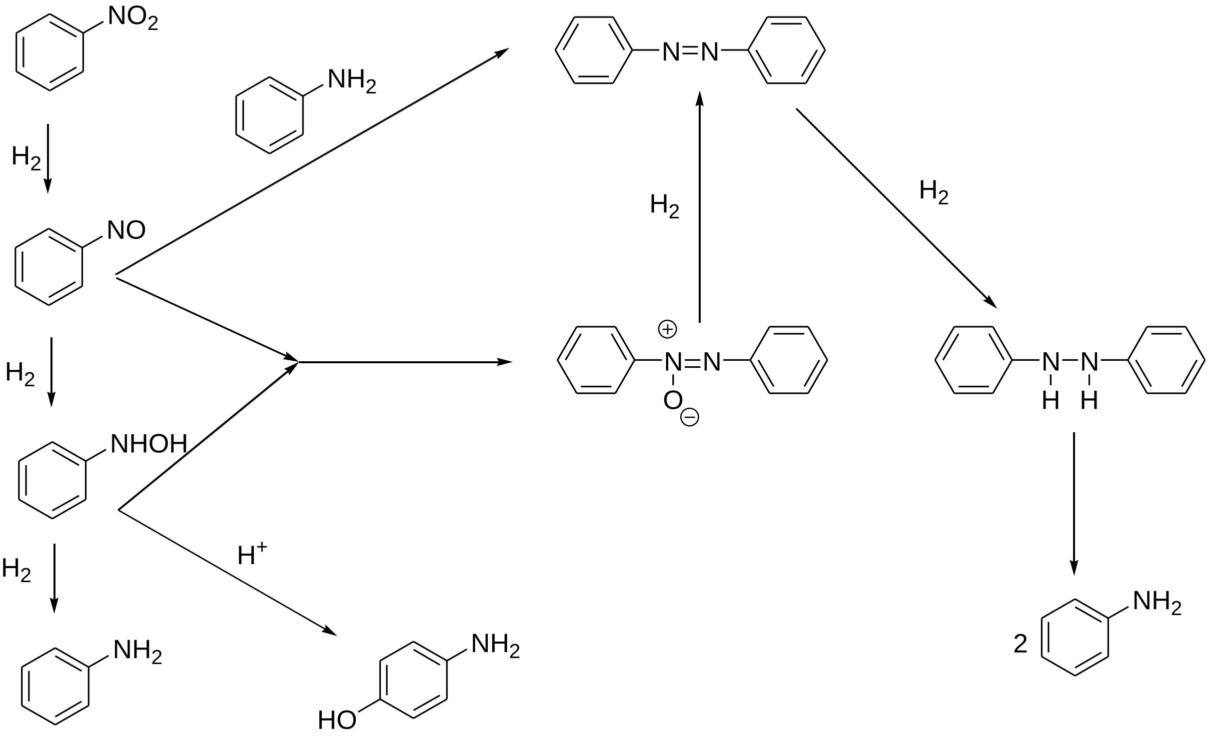
Table 3.6.4), so indicating the positive and synergic role of the iron species to favor the hydro-breaking of Ar-Cl bond[126] even if analytical studies exclude in these new catalysts the formation of alloy Fe-Pd (paragraph 3.1.2.3).

3.6.3 Final remarks

Homemade 0.28 % Pd/Al_2O_3 showed a high activity in hydrodechlorination reaction under mild conditions ($p(H_2)$ = 0.2 MPa and 30°C with substrate/catalyst molar ratio 100/1) in aqueous environment. However it was also observed that the catalyst, after the first use, was almost completely inactivated both in the presence of a base which can capture the produced HCl or in the absence of it. To verify the potentiality of this catalyst in the field of environmental remediation it will be necessary to understand the real role of the base and to investigate possible methods of protection of the catalyst. At the light of the obtained results in the reaction performed in neat conditions (run 3, Table 3.6.3,), appears interesting the possibility to operate directly in absence of solvent. It will also be important to verify the catalyst activity of homemade 0.28 % Pd/Al_2O_3 toward other substrates such as PCBs and similar polluting agents. Regarding the biogenerated catalysts it is possible to say that the presence of iron seems to have a beneficial effect on dehydrochlorination; in general, a partial activity of recovered catalyst was observed. In the future, it might be interesting to deep the influence of the base and other reaction parameters in order to increase the activity of recycled catalysts.

3.7 Reduction of aromatic nitro compounds: introduction

Aromatic nitro compounds are reduced to the corresponding amines which are utilized as important industrial intermediates for the synthesis of pigments, azo dyes, rubbers, amino-resins, herbicides and other fine chemicals. Over the years, many different methods to reduce nitro group have been developed such as reduction by using Fe in hydrochloric or acetic acid, by using sulphides, tin or stannous chloride in hydrochloric acid; however these methods are not sustainable due to the formation of waste sludge[129]. For these reasons, today nitroaromatic compounds are reduced to aromatic amines mainly by catalytic hydrogenation[130]. In this reaction, the oxygen atoms of the nitro group are progressively replaced by hydrogen; the reduction proceeds by formation of different intermediates. The currently most accepted reaction mechanism was proposed by Haber in 1898[129]:



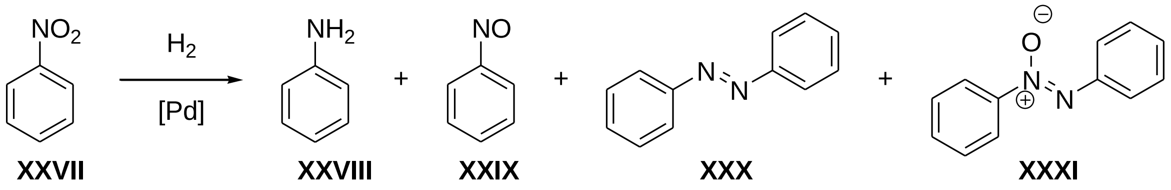
Scheme 3.7.1 Haber reduction mechanism for nitrobenzene

Therefore, at the light of the complexity of the reaction, it is possible to obtain many by-products if the reduction is performed by using inefficient catalysts. For these reason it was decided to study the activity and selectivity of different biogenerated catalysts (1.7 % $(Pd\text{-}EPS)_{aerob}$ and 8.4 %

Pd-Fe-EPS) and homemade 0.28 % Pd/Al_2O_3 in the hydrogenation of aromatic nitro-compounds to obtain the corresponding anilines.

3.7.1 Reduction of nitrobenzene (XXVII) catalysed by 1.7 % $(Pd\text{-}EPS)_{aerob}$ 8.4 % Pd-Fe-EPS and 0.28 % Pd/Al_2O_3

Aniline (**XXVIII**) is obtained by reduction of nitrobenzene (**XXVII**), and it is a very important industrial compound in different fields, especially for polymers, rubbers, agrochemicals, dyes and drugs industries. It is mainly obtained by reduction of **XXVII** although it can be produced by ammonolysis of chlorobenzene or phenol[130]. During this work of thesis, the reduction of **XXVII** was investigated by using three different homemade Pd-based catalysts as 1.7 % $(Pd\text{-}EPS)_{aerob}$, 8.4% Pd-Fe-EPS (Table 3.7.1), and 0.28% Pd/Al_2O_3 (Table 3.7.2).



Scheme 3.7.2. Reduction of nitrobenzene (XXVII)

Table 3.7.1. Reduction of nitrobenzene (XXVII) catalyzed by biogenerated Pd-based catalysts

Run	Catalyst	$p(H_2)$ MPa	T (°C)	t (h)	Conv. XXVII(%)	XXVIII (%)	XXIX (%)	XXX (%)	XXXI (%)	TOF
1[a]	Pd-EPS 1.7 %	5	80	24	98	98	---	---	---	40
2[a]	1° Recycling				99	99	---	---	---	41
3[a]	2° Recycling				99	99	---	---	---	41
4[a]	Pd-EPS 1.7 %	3	50	4	61	55	6	---	---	25
5[a]	1° Recycling				36	28	8	---	---	15
6[b]	Pd-Fe-EPS 8.4 %	3	80	5	83	67	10	1	5	83
7[b]	1° Recycling			20	51	7	41	1	2	13

Reaction conditions: [a] substrate **XXVII** = 0.120 g (0.00098 mol); 1.7 % Pd-EPS = 0.006 g (substrate **XXVII**/catalyst molar ratio 1000/1); THF = 2 mL, H_2O = 2 mL; [b] substrate **XXVII** = 0.246 g (0.002 mol); 8.4 % Pd-EPS = 0.005 g (substrate **XXVII**/catalyst molar ratio 500/1); THF = 4 mL; H_2O = 4 mL.

The first catalytic system applied in the hydrogenation of nitrobenzene was 1.7 % $(Pd\text{-}EPS)_{aerob}$. The reaction, carried out in the biphasic aqueous system H_2O/THF at 80°C and 5 MPa of H_2 for 24h, by using a substrate to palladium molar ratio 1000/1, afforded exclusively aniline (**XXVIII**) with 99% yield. After extraction of the organic products with diethyl ether, the catalyst was easily recovered by separation of the aqueous phase from the organic one and used in three consecutive recycling experiments without loss of activity (runs 2-3, Table 3.7.1). The reduction was also carried out lowering temperature, pressure and reaction time but a low conversion was observed (run 4) and when the recovered catalyst was re-used a significant decrease in activity was found (run 5). Preliminary hydrogenation experiments were carried out also in the presence of the bimetallic catalytic system 8.4 % Pd-Fe-EPS. The reaction was performed at 80°C and 3 MPa of hydrogen by using a substrate **XXVII**/catalyst molar ratio 500/1 in the aqueous biphasic system. Only after 5 h, 83 % conversion was observed but besides **XXVIII** (67 %), 10 % of nitrosobenzene (**XXIX**) and by-products (**XXX** and **XXXI**) were found.

The reduction of **XXVII** was also carried out in the presence of homemade 0.28 % Pd/Al_2O_3 and the obtained results are reported in Table 3.7.2.

Table 3.7.2. Reduction of XXVII catalysed by homemade 0.28 % Pd/Al_2O_3

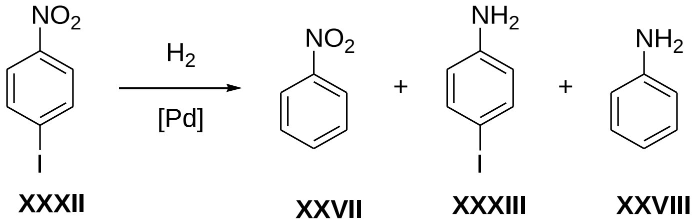
Run	$p(H_2)$ MPa	T (°C)	Solv	Sub/Cat m.r	t (h)	Conv XXVII(%)	XXVIII (%)	XXIXI (%)	XXX (%)	XXXI (%)	TOF
1[a]	3	80	toluene	500/1	20	99	99	---	---	---	25
2[a]	3	80	toluene	500/1	5	23	23	---	---	---	23
3[b]	3	80	THF	500/1	5	99	99	---	---	---	100
4[b]	0.5	50	THF	500/1	0.5	99	99	---	---	---	1000
5[b]	0.1	25	THF	500/1	20	99	99	---	---	---	25
6[c]	0.1	25	THF	100/1	1	99	99	---	---	---	500
7[c]	1° Recycling					99	99	---	---	---	500
8[c]	2° Recycling					90	90	---	---	---	455

Reaction conditions: [a] substrate **XXVII** = 0.016 g (0.13 mmol); 0.28 % Pd/Al_2O_3 = 0.01 g (substrate **XXVII**/catalyst molar ratio 500/1); toluene = 7 mL; [b] same conditions of run 1 but 7 mL of THF were used; [c] substrate **XXVII** = 0.065 g (0.53 mmol); 0.28 % Pd/Al_2O_3 = 0.2 g (substrate **XXVII**/catalyst molar ratio 100/1); THF = 10 mL.

Homemade 0.28 % Pd/Al_2O_3 catalyst showed excellent activity and selectivity in the reduction of **XXVII** operating under drastic conditions (runs 1 and 2, Table 3.7.2) or under milder conditions (runs 5 and 6, Table 3.7.2). Furthermore, it is also important to note that it was possible to recover the catalyst twice (run 8, Table 3.7.2) and both activity and selectivity remained almost unchanged.

3.7.2 Reduction of 1-iodo-4-nitrobenzene (XXXII)

When aromatic halonitro compounds are hydrogenated, some dehydrohalogenation is observed. The extent of dehalogenation depends on many factors: on the kind of halogen (I > Br > Cl > F) and its position on the molecule (ortho > para > meta), on the activity and amount of catalyst, on the kind of metal and support, on the kind of solvent, on pressure and temperature[129]. To improve the chemoselectivity of the reaction, during the past twenty years many different catalysts have been developed involving noble metals such as Pd, Pt, Ru and Rh and non-noble metals such as Fe, Co, Ni, Cu and Zn[131]. However, it is generally accepted that some commercially available Ni, Pd and Pt catalysts lack the necessary selectivity when substituted nitrobenzene are treated by H_2[132]. For these reasons, the search for new catalysts did not stop and for example Ru[133] or Co nanoparticles[131] as catalysts have been recently developed. At the light of these considerations, it was decided to test the selectivity of the homemade catalytic systems in the hydrogenation of a substrate containing both a nitro- and a halo-group: for this purpose 1-iodo-4-nitrobenzene (**XXXII**) as model substrate was chosen (Scheme 3.7.3, Table 3.7.3).



Scheme 3.7.3. Reduction of XXXII

Table 3.7.3. Reduction of XXXII catalysed by biogenerated 1.7 % (Pd-EPS)$_{aerob}$

Run	$p(H_2)$ (MPa)	T (°C)	Conv. XXXII (%)	XXVII (%)	XXXII (%)	XXVIII (%)	TOF
1	5	80	99	99	---	---	41
2	3	80	69	69	---	---	28
3	Recycling		26	26	---	---	11

Reaction conditions: substrate **XXXII** = 0.244 g (0.98 mmol); 1.7 % Pd-EPS = 0.005 g (substrate **XXXII**/catalyst molar ratio 1000/1); THF = 2 mL; H_2O = 2 mL.

A first reaction carried out in H_2O/THF at 80°C and 5 MPa for 24h, gave a complete conversion to **XXVII** but no product deriving from the hydrogenation of the nitro group was formed; by lowering the H_2 pressure to 3 MPa the only result was a lower conversion.

The inactivity of 1.7 % (Pd-EPS)$_{aerob}$ towards the reduction of the nitro group, at these reaction conditions, may be due to the formation of HI that could cause the deactivation of the catalytic system; therefore, to prevent the effect of HI the reaction was also carried out in an alkaline medium (Table 3.7.4) always 80°C and 3 MPa of H_2 for 24 h. By using an aqueous phosphate buffer (pH = 10) conversion was increased but, also in this case, only nitrobenzene (**XXVII**) was formed: when the catalytic phase was used in a recycling experiment, the conversion strongly decreased (Run 2, Table 3.7.4).

Table 3.7.4. Reduction of 1-iodo-4-nitrobenzene (XXXII) catalyzed by biogenerated 1.7 % (Pd-EPS)$_{aerob}$ and activated 1.7 % (Pd-EPS)$_{aerob}$ suspended in alkaline medium

Run	Cat.	T (°C)	Base	Conv. XXXII (%)	XXVII (%)	XXXIII (%)	XXVIII (%)	TOF
1[a]	Pd-EPS 1.7 %	80	pH 10 buffer	88	88	---	---	36
2[a]	1° Recycling			35	35	---	---	14
3[b]	Pd-EPS 1.7 %	80	Na_2CO_3	99	99	---	---	42
4[b]	1° Recycling			99	99	---	---	42
5[b]	2° Recycling			99	99	---	---	42
6[b]	Pd-EPS 1.7 %	80	Na_2CO_3	99	99	---	---	42
7[b]	1° Recycling			56	56	---	---	23
8[b]	Activated-Pd EPS 1.7 %	60	Na_2CO_3	98	98	---	---	41
9[b]	1° Recycling			42	42	---	---	18

Reaction conditions: [a] substrate **XXXII** = 0.244 g (0.98 mmol); 1.7 % Pd-EPS = 0.005 g (substrate **XXXII**/catalyst molar ratio 1000/1); THF = 2 mL; buffer (pH=10) = 2 mL; $p(H_2)$ = 3 MPa; t = 24 h; [b] 0.104 g (0.42 mmol) of Na_2CO_3 dissolved in 2 mL of water was used rather than pH=10 buffer.

The reaction was then carried out in the presence of Na_2CO_3 but also in this case only **XXVII** was obtained. The catalyst maintained its activity and selectivity in recycling experiment but the addition of fresh base to the catalytic solution was necessary. As a matter of fact, when the recycling experiment was carried out without the addition of fresh Na_2CO_3 solution, a strong activity decrease was observed, probably due to the free acidity in the reaction medium (run 7). Even when the hydrogenation of 1-iodo-4-nitrobenzene (**XXXII**) was performed by using activated 1.7 % (Pd-EPS)$_{aerob}$ as catalyst but working at 60°C and 3 MPa of H_2 for 24 h only formation of **XXVII** (98 %) was detected (run 8, Table 3.7.4). The aqueous catalytic phase was recycled without adding any fresh base and again the catalytic activity decreased, so confirming that the fresh base must be added to preserve the activity of the catalyst.

The reaction was also studied by using 8.4 % Pd-Fe-EPS, initially in the absence of a base and the obtained results are reported in Table 3.7.5.

Table 3.7.5. Reduction of XXXII catalyzed by Pd-Fe-EPS 8.4 %

Run	Conv. XXXII (%)	XXVII (%)	XXXIII (%)	XXVIII (%)	TOF
1	86	85	1	1	21
1° recycle	91	90	---	1	22
2° recycle	56	53	2	1	12
3° recycle	24	19	5	---	5

Reaction conditions: substrate **XXXII** = 0.488 g (0.00196 mol); 8.4 % Pd-Fe-EPS = 0.005 g (substrate **XXXII**/catalyst molar ratio 500/1); THF = 5 mL; H_2O = 5 mL; p(H_2) = 3 MPa; T = 80°C; t = 24 h.

All the experiments were carried out at 3 MPa of H_2 and 80°C for 24h by using a substrate **XXXII**/catalyst molar ratio 500/1. In the presence of fresh catalyst and in the first recycle experiment a good activity was observed (run 1 and first recycling test, Table 3.7.5), but during the second and the third recycle a decrease in activity was detected, probably due to the increased amount of HI in the aqueous phase. In fact, before each recycling, pH was checked and it was found that it was acid. Noteworthy, the formation of small amounts of **XXXIII** and **XXVIII** was detected (run 1, Table 3.7.5) either in the presence of fresh catalyst or in all the recycling experiments.

Then, the reduction was carried out by using an organic base in order to try to maintain good catalytic activity also in the recycling experiments (Table 3.7.6).

Table 3.7.6. Reduction of XXXII catalyzed by Pd-Fe-EPS 8.4 % in presence of trioctylamine

Run	Conv. XXXII (%)	XXVII (%)	XXXIII (%)	XXVIII (%)	TOF
1	88	88	---	---	22
1° Recycle	85	85	---	---	21
2° Recycle	80	80	---	---	19
3° Recycle	54	54	---	---	15
4° Recycle	52	52	---	---	14

Reaction conditions: substrate **XXXII** = 0.488 g (0.00196 mol); 8.4 % Pd-Fe-EPS = 0.005 g (substrate **XXXII**/catalyst molar ratio 500/1); TOA = 2.6 mL (base/substrate **XXXII** molar ratio 3/1, d = 0.809 g/mL); THF = 5 mL; H_2O = 5 mL; p (H_2) = 3 MPa, T = 80°C; t = 24 h.

Operating under the conditions reported in Table 3.7.6, it was observed that the selectivity of the catalyst was improved with respect to the reaction without the base (table 3.7.5). However, despite the addition of the base, nitrobenzene was the only product both in the presence of fresh and recycled catalyst. Noteworthy the catalytic activity remained practically unchanged in two recycling experiments but a conversion decrease was observed starting from the third recycle.

The only formation of **XXVII** could be due to traces of salt of TOA in aqueous phase that could deactivate the catalyst.

Also the homemade 0.28 % Pd/Al_2O_3 was investigated and analogously to the other catalysts its activity was tested in some recycling experiments (Table 3.7.7).

Table 3.7.7. Reduction of XXXII catalyzed by 0.28 % Pd/Al_2O_3

Run	Conv. XXXII (%)	XXVII (%)	XXXIII (%)	XXVIII (%)	TOF
1[a]	99	99	---	---	21
1° Recycle	99	99	---	---	21
2° Recycle	66	63	3	---	14
2[b]	---	---	---	---	---

Reaction Conditions: [a] substrate **XXXII** = 0.488 g (0.00196 mol); 0.28 % Pd/Al_2O_3 = 0.148 g (substrate **XXXII**/catalyst molar ratio 500/1); H_2O = 10 mL; p (H_2) = 3 MPa; T = 80°C; t = 24 h, [b] same conditions of run 1 but 0.074 g of 0.28 % Pd/Al_2O_3 were used (substrate **XXXII**/catalyst molar ratio 1000/1).

Since this catalyst is active in hydrodechlorination using water as solvent, it was decided to study the reaction in aqueous phase. When the reaction was carried out using a substrate **XXXII**/catalyst molar ratio 500/1 instead of 1000/1, quantitative substrate **XXXII** conversion was found but again **XXVII** was the only reaction product. 0.28 % Pd/Al_2O_3 was recovered and recycled twice: in the first recycle, quantitative conversion to nitrobenzene **XXVII** was found but in the second recycle a small decrease in catalytic activity was detected (2° recycle, Table 3.7.7). Furthermore a small amount of **XXXIII** was found in the reaction mixture.

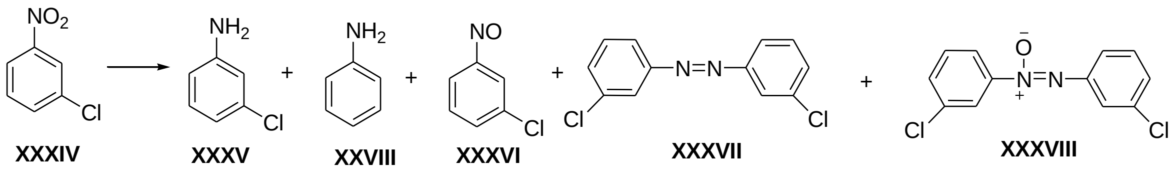
In general, surprisingly, the reduction of the nitro group was never observed but only dehalogenation was detected under the adopted conditions. It is possible to hypothesize a detachment of palladium from the support, followed by a re-absorption of the metal; however, reabsorbed Pd, although partially active, is not able to reduce the nitro groups. In fact, by Atomic Absorption analysis, no leaching was detected.

3.7.3 Reduction of 1-chloro-3-nitrobenzene (XXXIV)

Another substrate investigated during this work of thesis was 1-chloro-3-nitrobenzene (**XXXIV**). By reduction of **XXXIV** is possible to obtain 3-chlorobenzenamine (**XXXV**), better known as m-chloroaniline or 3-chloroaniline. This compound is an intermediate for dyes, drugs, and pesticides[134]. An important application **XXXV** is also in the manufacture of antimalarial drugs such as chloroquine and amodiquin[135].

In the hydrogenation of **XXXIV** is desirable to obtain the selective hydrogenation of the nitro-group, without dehalogenation. This selectivity depends on the kind of catalyst used and on the reaction conditions. In order to reduce the by-side reactions (essentially hydrodechlorination) often poisoned catalysts were adopted[129,136]. Recently good results in selective hydrogenation **XXXIV** with hydrazine have been reported by using a Co catalyst modified with Mo-carbide[131] or with isopropanol by using Ru nanoparticles stabilized on modified Montmorillonite clay[133].

Since homemade 0.28 % Pd/Al_2O_3 is simple to prepare and showed a good activity towards both the reduction of nitro group and the hydrodechlorination reaction, it was decided to study the activity and selectivity of this catalytic system in the reduction of **XXVIII** chosen as model substrate (Table 3.7.8).



Scheme 3.7.4. Reduction of 1-chloro-3-nitrobenzene (XXXIV)

Table 3.7.8. Reduction of (XXXIV) catalyzed by 0.28 %Pd/Al_2O_3

Run	$p(H_2)$ MPa	T (°C)	S/C m.r	Conv. (XXXIV) (%)	XXXV (%)	XXVIII (%)	XXXVI (%)	XXXVII (%)	XXXVIII (%)	TOF
1[a]	3	80	100/1	99	66	33	---	---	---	5
2[a]	0.2	30	100/1	99	87	5	7	---	---	5
3[a]		Recycle		99	91	4	4	---	---	5
4[b]	3	80	500/1	99	95	4	---	---	---	20
5[b]		Recycle		99	90	9	---	---	---	20
7[c]	5	50	100/1	99	80	19	---	---	---	100
8[c]		Recycle		---	---	---	---	---	---	100
9[d]	5	50	500/1	90	84	6	---	---	---	500
10[d]		Recycle		81	72	6	3			500

Reaction conditions: [a] substrate **XXXIV** = 0.077 (0.00049 mol); 0.28 % Pd/Al_2O_3 = 0.200 g; THF = 10 mL; [b] substrate **XXXIV** = 0.385 g (0.0024 moli); 0.28 % Pd/Al_2O_3 = 0.200 g (substrate **XXXIV**/catalyst molar ratio 500/1); THF = 20 mL; t = 20 h; [c] same conditions of run 1, but the reaction was carried out for 1 h; [d] same conditions of run 4, but the reaction was carried for 1 h.

At the light of obtained results, it is possible to observe that working at high pressure and high substrate **XXXIV**/catalyst molar ratio the dechlorination is favored with respect to use milder conditions (run 1 vs runs 2 and 9, Table 3.7.8). The recycling of the catalyst was possible; however it is noteworthy that the formation of HCl leads to the deactivation of the catalyst, especially if the acid amount is high (run 7, Table 3.7.8).

The reaction was also investigated by introducing $NaHCO_3$ in the reaction mixture with a base/substrate **XXXIV** molar ratio 3/1, but a high grade of dechlorination was observed (Table 3.7.9).

Table 3.7.9. Reduction of XXXIV catalyzed 0.28 %Pd/Al_2O_3 in presence of $NaHCO_3$

Run	Time	Conversion XXXIV (%)	XXXV (%)	XXVIII (%)	XXXVI (%)	XXXVII (%)	XXXVIII (%)
1[a]	2	43		15			28
	4	94		47			47
	6	94		55			39
	24	94		81			13
2[a]	1	99		99	---	---	---
1°Recycle[b]		95		95	---	---	---
2°Recycle[b]		73	18	23	3	---	18

Reaction conditions: [a] substrate **XXXIV** = 0.035 g (0.00022 mol); 0.28 % Pd/Al_2O_3 = 0.090 g (substrate **XXXIV**/catalyst molar ratio 100/1); $NaHCO_3$ = 0.055 g (base/substrate **XXXIV** molar ratio 3/1); p(H_2) = 0.2 MPa; T = 30°C; THF = 10 mL; [b] substrate **XXXIV** = 0.070 g (0.00044 mol); 0.28 % Pd/Al_2O_3 = 0.180 g (substrate **XXXIV**/catalyst molar ratio 100/1); $NaHCO_3$ = 0.110 g (base/substrate **XXXIV** molar ratio 3/1); p(H_2) = 0.5 MPa; T = 50°C; THF = 10 mL.

Operating at 50°C and 0.5 MPa of H_2 (run 2, Table 3.7.8) after only 1 h, the complete formation of aniline (**XXVIII**) was detected. This result is not surprising as this catalyst is also active in the hydrodechlorination reaction and probably in the presence of a base this reaction becomes competitive with the hydrogenation of the nitro group[136]. However, it is interesting to note that the catalyst was recycled twice and in the first recycling test only a slight decrease in activity was detected.

The reaction was also studied using 8.4 % Pd-Fe-EPS as catalyst:

Table 3.7.10 Reduction of 1-chloro-3-nitrobenzene(XXXIV) catalyzed by 8.4 %Pd-Fe-EPS

Run	Conv. XXXIV(%)	XXXV (%)	XXXVII (%)	XXXVIII (%)	TOF
1[a]	81	47	9	25	20
1° Recycle	75	58	5	22	19
2° Recycle	69	45	3	21	18

Reaction conditions: substrate **XXXIV** = 0.189 g (0.0012 mol); 8.4 % Pd-Fe-EPS = 0.003 g (substrate **XXXIV**/catalyst molar ratio 500/1); THF = 3 mL; H_2O = 3 mL; p(H_2) = 3 MPa; T = 80°C; t = 20 h.

Performing the reaction by using this catalyst, incomplete conversion and low selectivity were detected under drastic conditions and long reaction times. However, the catalyst showed a good recyclability. At the light of these results, this catalyst does not appear so promising for this reaction; however, it is important to note that no dehalogenation was observed.

Recently the reaction was also performed by using homemade 0.27 % Pt/Al_2O_3. Operating at 50°C, 0.5 MPa of hydrogen and with substrate **XXXIV**/catalyst molar ratio 100/1, after 7 h, 94 % conversion of **XXXIV** was detected. The reaction is characterized by high selectivity in fact, no dehalogenation products were observed (80 % **XXXV**, 7 % **XXXVII,** 7 % **XXXVIII**)

3.7.4 Conclusions

Homemade 0.28 %Pd/Al_2O_3, 1.7 % $(Pd\text{-}EPS)_{aerob}$ and 8.4 % Pd-Fe-EPS showed a good activity and selectivity in the hydrogenation of nitro group, however when a halo atom is present on the aromatic ring, different behaviors were observed (Table 3.7.10).

Table 3.7.11. Comparison among homemade catalysts

Reaction	Catalyst	Observation
Reduction of nitrobenzene (XXVII)	1.7 % $(Pd\text{-}EPS)_{aerob}$	High selectivity, no formation of intermediate, recycling is possible
	8.4 % Pd-Fe-EPS	Good selectivity, after 20 h, small amounts of intermediate was detected, recycling is possible but decrease of activity is observed
	0.28 % Pd/Al_2O_3	High activity under mild conditions, recycling is possible
Reduction of 1-iodo-4-nitrobenzene (XXXII)	1.7 % $(Pd\text{-}EPS)_{aerob}$	High activity in deiodination reaction, activity is highly preserved when the base is added
	8.4 % Pd-Fe-EPS	High activity in deiodination reaction, activity is highly preserved when the base is added, aniline formation was not observed.
	0.28 % Pd/Al_2O_3	High activity in deiodination reaction, recycling is possible, working in water system, aniline formation was not detected.
Reduction of 1-chloro-3-nitrobenzene (XXXIV)	8.4 % Pd-Fe-EPS	The reaction is slow, this catalyst does not appear interesting in this reaction.
	0.28 % Pd/Al_2O_3	High activity in the selective reduction of nitro-group: high formation of 3-chlorobenzamine; recycling is possible.
	0.27 % Pt/Al_2O_3	Good activity in the selective reduction of nitro-group: hydrodechlorination was not observed.

At the light of obtained results, homemade catalysts appear interesting for the reduction of aromatic nitro-group and seem attractive for a possible future application for other substrates of industrial interest.

3.8 Hydrogenation of α-β-unsaturated aldehydes

The regioselective hydrogenation of α,β-unsaturated aldehydes to the corresponding saturated aldehydes or to the unsaturated alcohols is a very relevant reaction for the synthesis of valuable fine chemicals. The aim was to study the selective reduction of C=C double bonds of some unsaturated aldehydes (Figure 3.8.1).

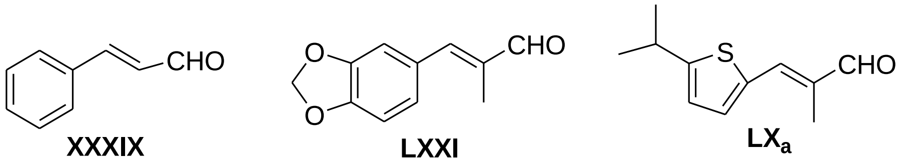
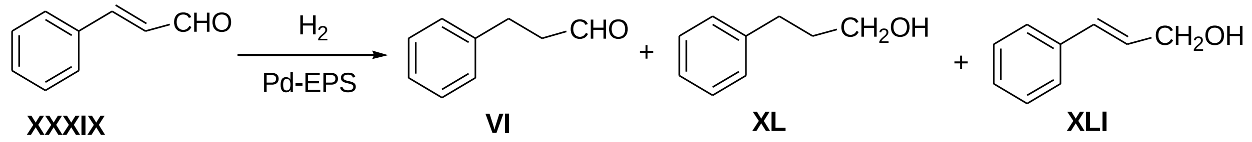


Figure 3.8.1. α,β-unsaturated aldehydes investigated during this work of thesis

In particular, in this paragraph 3.8, the selective reduction of C=C double bond of *trans*-cinnamaldehyde (**XXXIX**) with homemade Pd and Rh based catalysts is described, and, for comparison, results obtained are also reported with some commercial catalysts. According to the type of used catalysts, different solvents were tested and the influence of medium on activity and selectivity was investigated.

The regioselectivity of the reaction is influenced by the nature of the catalytically active metal and by the used experimental conditions[137,138,139,140]. For example, in the literature it is reported that heterogeneous palladium-based catalysts are very selective towards the C=C double bond hydrogenation[141], while water-soluble Ru(II)-phosphane complexes at pH 3.04 (phosphate buffer), produce a 93:7 mixture of cinnamyl alcohol and dihydrocinnamaldehyde at 0.8 MPa of H_2[142].

Moreover in the literature, water-soluble catalysts operating in aqueous biphasic system are able to reduce selectively the C=C double bond of crotonaldehyde. However, when the reaction is carried out on **XXXIX**, the simultaneous hydrogenation of the carbonyl group and the C=C double bond was observed with the prevailing formation of 3-phenylpropan-1-ol (**XL**)[143]. On the base of these results, the activity and selectivity of the catalysts 1.7 % (Pd-EPS)$_{aerob}$ and the corresponding activated 1.7 % (Pd-EPS)$_{aerob}$ were investigated in the aqueous biphasic hydrogenation of **XXXIX** (Table 3.8.1).



Scheme 3.8.1. Hydrogenation of XXXIX catalyzed by 1.7 % (Pd-EPS)$_{aerob}$ and activated 1.7 % (Pd-EPS)$_{aerob}$

Table 3.8.1. Hydrogenation of trans-cinnamaldehyde (XXXIX) catalyzed 1.7 % (Pd-EPS)$_{aerob}$ and activated 1.7 % (Pd-EPS)$_{aerob}$

Run	Catalyst	Conv.XXXIX (%)	VI (%)	XL (%)	XLI (%)	TOF
1[a]	**1.7% Pd-EPS**	63	48	12	3	103
	1° Recycle	56	43	9	4	91
	2° Recycle	55	45	8	2	90
	3° Recycle	54	44	8	2	89
2	**Activated 1.7% Pd-EPS**	96	89	6	1	157
	1° Recycle	94	89	5	Traces	154
	2° Recycle	93	88	4	1	153
	3° Recycle	99	93	6	Traces	162

[a]Reaction condition: substrate **XXXIX** = 0.26 g (0.00196 mol); 1.7 % (Pd-EPS)$_{aerob}$ or 1.7 % activated (Pd-EPS)$_{aerob}$ = 0.012 g (substrate **XXXIX**/catalyst molar ratio 1000/1); THF = 4 mL; H_2O = 4 mL, T = 50°C; p(H_2) = 2 MPa; t = 6 h

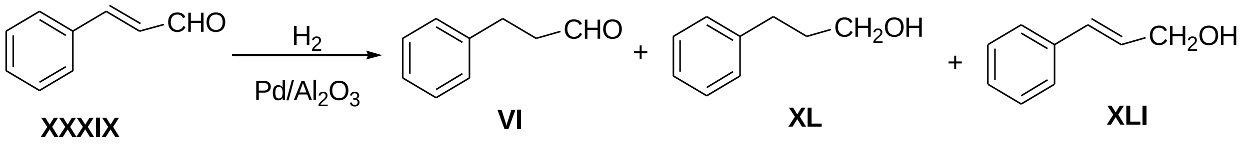
As showed in the table 3.8.1, when 1.7 % (Pd-EPS)$_{aerob}$ was used, conversion was fairly good (63%) and besides 3-phenylpropanal (**VI**), the main reaction product, also 3-phenylpropan-1-ol (**XL**) (12 %) and 3-phenylprop-2-en-1-ol (**XLI**) (3 %) were formed (run 1, Table 3.8.1). Furthermore, it is interested to note that the aqueous catalytic phase was recycled three times and its activity and selectivity remained practically unchanged.

Much better results, either as conversion (up to 99 %) or selectivity towards the C=C double bond hydrogenation (up to 93 %), were found using activated 1.7 % (Pd-EPS)$_{aerob}$ (run 2, Table 3.8.1).

Analogously to 1.7 % $(Pd\text{-}EPS)_{aerob}$, also activated 1.7 % $(Pd\text{-}EPS)_{aerob}$ maintained its activity in three consecutive recycling experiments.

When the reaction was performed in THF as reaction solvent, only 4 % conversion was found, so further confirming that water is essential for the activity of this type of catalyst.

On the same substrate also the activity of homemade 0.28 % Pd/Al_2O_3 was investigated (Scheme 3.8.2; Table 3.8.2).



Scheme 3.8.2. Hydrogenation of trans-cinnamaldehyde (XXXIX) catalyzed by 0.28 % Pd/Al_2O_3

Table 3.8.2. Hydrogenation of trans-cinnamaldehyde (XXXIX) catalyzed by 0.28 %Pd/Al_2O_3

Run[a]	$p(H_2)$ MPa	T (°C)	Conv.XXXIX (%)	VI (%)	XL (%)	XLI (%)	TOF
1	0.5	50	99	89	10	---	126
2	0.2	50	99	96	3	---	126
3	0.5	35	99	96	3	---	126
4[b]	0.5	35	40	40	---	---	58

[a]Reaction conditions: substrate **XXXIX** = 0.09 g (0.00068 mol); 0.28 % Pd/Al_2O_3 = 0.01 g (substrate **XXXIX**/catalyst molar ratio 2500/1); toluene = 8 mL; t = 20 h; [b] reaction carried out for only 6 h.

The catalyst showed high activity and selectivity towards the C=C double bond hydrogenation under mild conditions.

Adopting the comparative experiment by using the commercial catalyst 5 % Pd/C (Chimet), that is used on industrial level with good success in many processes, after 20 h only 20 % of saturated aldehyde **VI** was formed.

Very interestingly, by using a 0.5% Pd/Al_2O_3 catalyst (generous gift of Università di Pisa), obtained by using a microwave technique for the preparation but the same γ-alumina used by us as support a slight improvement of activity and selectivity was observed after 5 hours of reaction (result not reported here) with respect to the results obtained by using our homemade 0.28 % Pd/Al_2O_3. This result could be explained by assuming a better dispersion of the metal due to the effect of microwaves, but it also worthy to note the importance of this alumina support in the preparation of these catalysts.

Finally the influence of the stirring method (mechanical or magnetic stirrer) on the reaction was investigated: the reaction was carried out on a larger scale in order to easier recover and recycle homemade 0.28 % Pd/Al_2O_3. The obtained results are reported in Table 3.8.3.

Table 3.8.3. Hydrogenation of XXXIX by using 0.28 % Pd/Al_2O_3 under mechanical stirrer

Run	Conv. XXXIX (%)	VI (%)	XL (%)	XLI (%)	TOF
1[a]	99	85	14	---	125
1° Recycle[b]	99	85	14	---	125
2° Recycle[c]	99	82	17	---	417

Reaction conditions: substrate **XXXIX** = 6 g (0.045 mol); 0.28 % Pd/Al_2O_3 = 0.684 g (substrate **XXXIX**/catalyst molar ratio 2500/1); toluene = 60 mL; T = 35°C; $p(H_2)$=0.2 MPa; t = 20 h; [b] Experiment carried out with recovered catalyst from previous test; [c] reaction time = 6 hours.

The catalytic system showed a great activity, also in two consecutive recycling experiments (Table 3.8.3) but a decrease of selectivity was observed. These results highlight the necessity of a fine tuning of reaction parameters, especially stirring mode and shape of reactor, when the scale of reaction is increased. Further studies using bigger equipment, not available at university, should be carried out in order to optimize both activity and selectivity of this catalyst. In any case this catalyst shows promising performances and very good stability.

The hydrogenation of **XXXIX** was carried out also in the presence of other two commercial catalysts claimed by the supplier (BASF SE) as very active catalytic systems in this type of reaction.

These catalysts called Nanoselect LF 100 (BASF 0.6 %) and Nanoselect LF 200 (BASF 0.5 %) respectively, are prepared by reduction-deposition technology. This procedure is quite similar to the one used in the preparation of homemade heterogeneous catalyst but a relevant difference is the nature of support and the fact that the preparation of catalyst is made in water in the presence of a surfactant. By this technology the metal crystallite size is independent of support and it does not change after first formation:

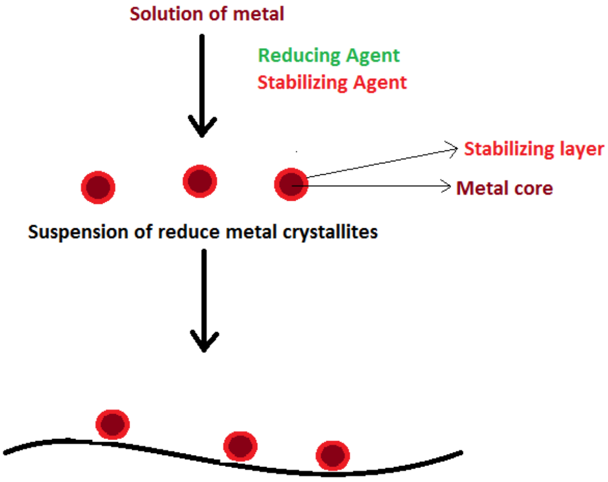
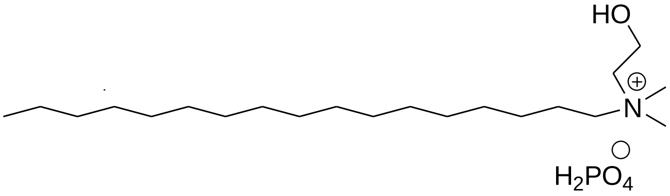


Figure 3.8.2 Preparation of BASF catalysts

The metal salt is reduced and stabilized in one step by using HHDMA (Hexadecyl(2-hydroxyethyl)-dimethylammonium-dihydrogenphosphate):

$$Metal\ \ salt + HHDMA\ \ \rightarrow metal\ colloid$$



HHDMA

Figure 3.8.3. The structure of HHDMA

The obtained metal-colloid was deposed on carbon (BASF 0.5 %) or on titaniumsilicalite (BASF 0.6 %). The procedure for the preparation of colloidal nanoparticles and deposit them on different supports is described in the patent[144] .

These catalysts were used under the conditions reported in Table 3.8.4.

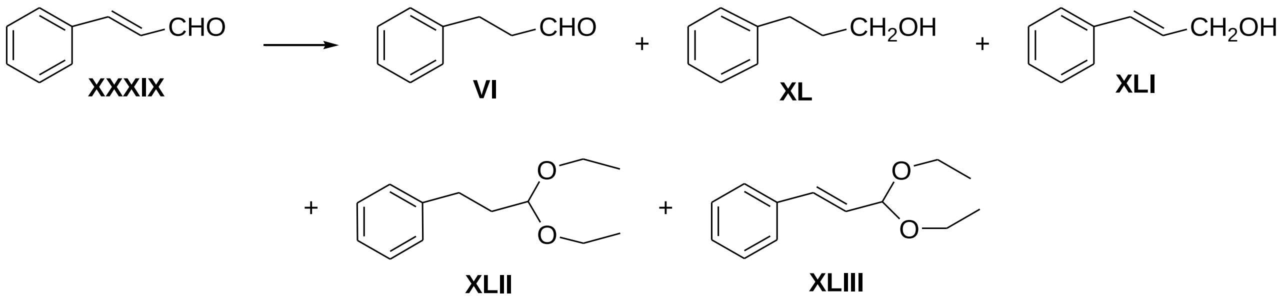
Table 3.8.4. Hydrogenation of XXXIX catalyzed by 0.5 % and 0.6 % BASF catalysts

Run	Catalyst	$p(H_2)$ MPa	Conv. XXXIX (%)	VI (%)	XL(%)	XLI (%)
1[a]	BASF 0.5 %	0.2	**No reaction**			
2[a]		1				
3[b]	BASF 0.6 %	0.2				
4[b]		1				

Reaction conditions: [a] substrate **XXXIX** = 0.150 g (0.0011 mol); catalyst = 0.013 g (substrate **XXXIX**/catalyst molar ratio 2500/1, 20 % wet); T = 35 °C; t = 20 h. [b] Same conditions of run 1 but 0.019 g of catalyst was used (substrate **XXXIX**/catalyst 2500/1, 56 % wet).

The reaction was first carried out by using both catalysts at the same reaction conditions previously applied with success (Table 3.8.2), in the presence of homemade 0.28 % Pd/Al_2O_3 catalyst; unfortunately any reaction did not occur. The same disappointing result was obtained also by using the BASF catalysts activated by a pre-treatment with hydrogen (0.2-1 MPa overnight at room temperature). At the light of these results, it was decided to carry out the reaction in the presence of the BASF catalysts, on homemade 0.28 % Pd/Al_2O_3 and the catalyst prepared at Università of Pisa but adopting the experimental conditions suggested by BASF SE[145].

The obtained results are reported in the Table 3.8.5.



Scheme 3.8.3. Hydrogenation of XXXIX by using BASF conditions

Table 3.8.5. Hydrogenation of XXXIX by using BASF SE conditions and under mechanical stirring

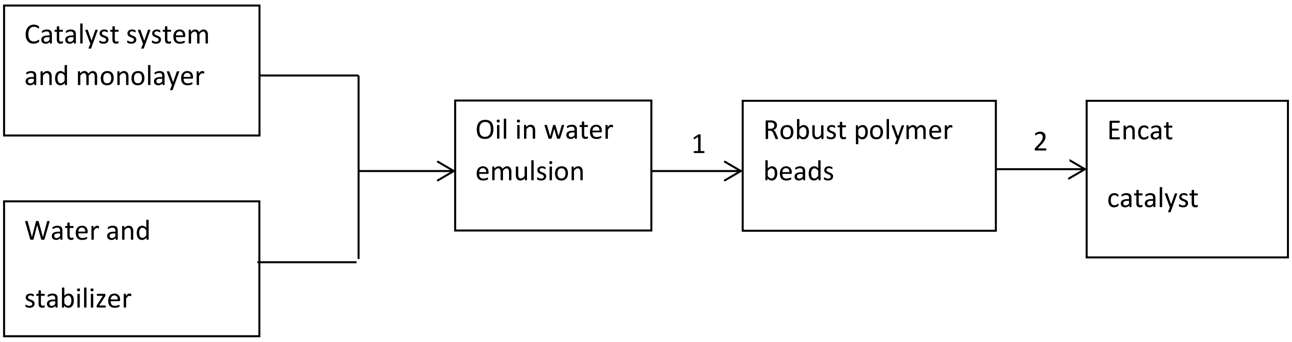
Run	Cat	T (h)	Conv. XXXIX (%)	VI (%)	XL (%)	XLI (%)	XLII (%)	XLIII (%)	TOF
1[a]	BASF 0,5 %	1	---	---	---	---	---	---	---
2[b]	BASF 0,5 %	20	30	9	---	---	3	18	241
3[c]	BASF 0,6 %	20	46	8	---	---	11	27	739
4[d]	Pd/Al_2O_3 0,28 %	2	92	14	8	---	67	3	14786
5[e]	Pd/Al_2O_3 PISA	2	95	53	5	---	37	---	15267

Reaction conditions: [a] substrate **XXXIX** = 6 g (0.045 mol); BASF 0.5 % catalyst = 0.036 g (substrate **XXXIX**/catalyst molar ratio 32000/1, 20 % wet); T = 60°C; $p(H_2)$ = 2 MPa; ethanol = 94 mL; [b] Same conditions of run 1 but substrate **XXXIX** molar ratio was 16000/1; [c] Same conditions of run 1 but 0.057 g of BASF 0.6 % (substrate **XXXIX**/catalyst molar ratio 32000/1, 56 % wet) was used; [d] Same conditions of run1 but 0.053 g of 0.28 % Pd/Al_2O_3 (substrate **XXXIX**/catalyst molar ratio 32000/1) was used; [e] Same conditions of run 1 but 0.030 g of 0.5 % Pd/Al_2O_3 PISA was used.

The reactions were carried out in ethanol by using a substrate **XXXIX**/catalyst molar ratio 32000/1 (with the exception of run 2, where the substrate **XXXIX**/catalyst molar ratio was 16000/1). When homemade 0.28 % Pd/Al_2O_3 catalyst was used, a high conversion of substrate **XXXIX** was found but selectivity was rather low. In fact, a high formation of acetal **XLII** was detected in the reaction mixture (run 4, Table 3.8.5). Operating in the presence of the catalyst prepared at Università di Pisa, activity was always high and curiously, acetal **XLII** was formed in a lower amount, probably microwave slight affect the characteristics of alumina.

The formation of saturated and unsaturated acetals (**XLII** and **XLIII**) is probably due to the presence of Brønsted acid sites on alumina, (see paragraph 3.1.1.2). In support of this hypothesis the reaction of **XLIII** in ethanol was carried out in the presence only of γ-alumina and the unsaturated acetal **XLIII** was formed. It is important to remark that, according to the observed results, homemade 0.28 % Pd/Al_2O_3 and the catalyst supplied by Università di Pisa showed a superior activity compared to the catalysts commercialized by BASF SE.

The hydrogenation of trans-cinnamaldehyde (**XXXIX**) was finally investigated using another commercial catalyst, 4.3 % Pd-ENCAT, an encapsulated homogeneous palladium phosphine catalyst. In fact the metal and the phosphine ligand are entrapped into a polymeric matrix (polyurea) and this feature, compared to homogeneous catalysts, permit a low metal contamination on the final product, an easy filtration and a possible re-use of the catalyst, also allowing a flow process. The method of preparation is shown schematically below:



1 = initiation of polymerization

2 = onward processing and purification

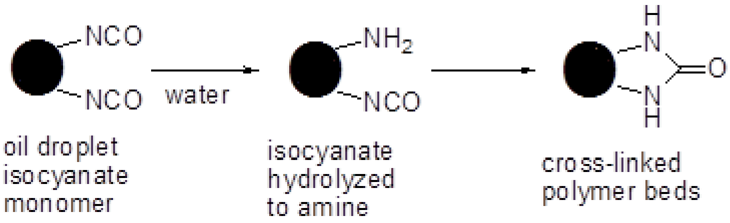
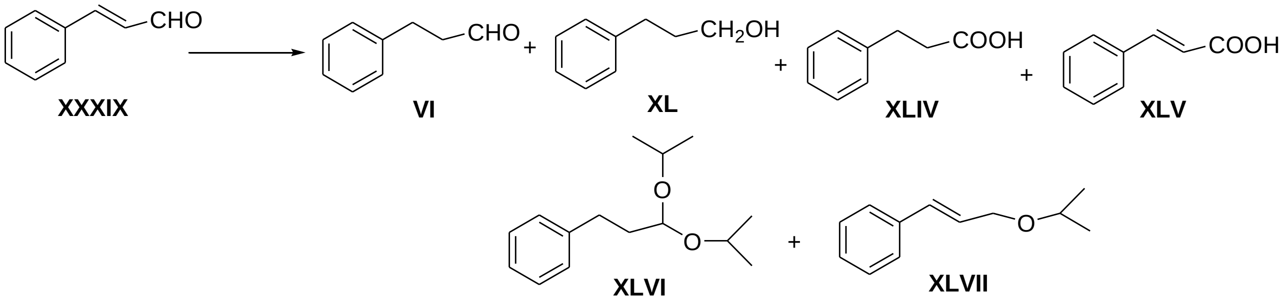


Figure 3.8.3. Preparation of 4.3 %Pd-ENCAT[146]

This technology allows the encapsulation of a range of catalytic systems within a polymeric matrix. The polymer coordinates the metal so retaining it into the matrix. Furthermore, the metal is equally distributed throughout the polymer bead. The particle sizes are controlled and the highly porous matrix allows a good diffusion of substrates in and out of the beads.

This catalyst was used under different conditions summarized in Table 3.8.6.



Scheme 3.8.4. Hydrogenation of trans-cinnamaldehyde (XXXIX) by using Pd-ENCATS 4.3 %

Table 3.8.6. Hydrogenation of XXXIX catalyzed by 4.3 % Pd-ENCATS

Run	Conv. XXXIX(%)	VI (%)	XL (%)	XLIV (%)	XLV (%)	XLVI (%)	XLVII (%)	Others by-products (%)	TOF
1[a]	19	19	---	---	---	---	---	---	1
2[b]	99	52	11	6	---	28	---	2	5
3[c]	97	36	24	2	---	12	4	19	2
4[d]	98	58	8	7	---	19	4	2	4
5[e]	44	26	---	11	7	---	---	---	2

Reaction conditions: [a] substrate **XXXIX** = 0.533 g (0.004 mol); 4.3 % Pd-ENCAT = 0.100 g (substrate **XXXIX**/catalyst molar ratio 1000/1), toluene = 10 mL, p(H_2)= 0.2 Mpa; T = 40°C; t = 20 h; [b] Same conditions of run 1 but p(H_2)= 0.5 MPa; mixture of toluene/propan-2-ol 8.5/1.5 = 10 mL; [c] same conditions of run 1 but 0.200 g of 4.3 % Pd-ENCAT was used (substrate **XXXIX**/catalyst molar ratio 500/1); mixture of toluene/propan-1-ol 8.5/1.5 = 10 mL; [d] Same conditions of run 1 but the solvent was 10 mL mixture of toluene/propan-1-ol 8.5/1.5; [e] same conditions of run 1 but the 10 mL of Ethyl Acetate as solvent was used.

Due to the low selectivity obtained by using this catalyst, it is possible to conclude that 4.3 % Pd-ENCAT is not suitable for this type of reaction.

Finally, the reduction of trans-cinnamaldehyde (**XXXIX**) was also performed by using homemade 0.18 % Rh/Al_2O_3. Preliminary results are reported in Table 3.8.7.

Table 3.8.7. Hydrogenation of XXXIX catalyzed by homemade 0.18 % Rh/Al_2O_3

Run	Sub/cat molar ratio	Conv. XXXIX (%)	VI (%)	XL (%)	XLI (%)	XLII (%)	XLIII (%)	TOF
1[a]	1000/1	99	99	---	---	---	---	254
2[b]	3800/1	40	40	---	---	---	---	1553
3[c]	32000/1	58	4	1	---	21	32	4179

Reaction conditions: [a] substrate **XXXIX** = 0.088 g (0.66 mmol); 0.18 % Rh/Al_2O_3 = 0.010 g; toluene = 10 mL; p(H_2) = 0.5 MPa; T = 50°C; t = 4 h; [b] substrate **XXXIX** = 0.023 g (0.174 mmol); 0.18 % Rh/Al_2O_3 = 0.010 g; toluene = 10 mL; p(H_2) = 0.5 MPa; T = 50°C; t = 1 h; [c] substrate **XXXIX** = 6 g (0.045 mol); 0.18 % Rh/Al_2O_3 = 0.036 g; p(H_2) = 2 MPa; T = 60°C; ethanol = 94 mL, t = 2 h.

Also working in the presence of this catalyst, excellent conversion and selectivity were found operating under mild conditions. Further investigations are necessary to evaluate the real potentiality of this catalyst and its recyclability. Therefore, at the moment, it is not possible to establish which catalyst (0.18 % Rh/Al_2O_3 or 0.28 % Pd/Al_2O_3) is more sustainable for this reaction.

3.8.1 Conclusive remarks

The most significant results are summarized in Table 3.8.8 reported below.

Table 3.8.8. Summary of the results and possible future developments

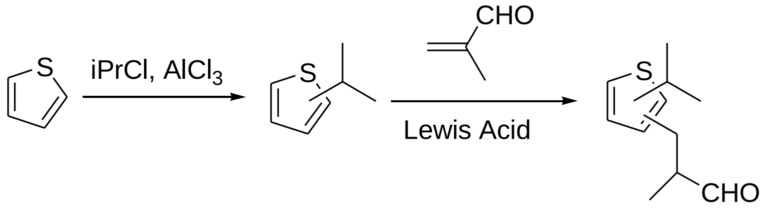
Catalyst	Operation conditions	Results	Future development
1.7 % $(Pd\text{-}EPS)_{aerob}$ and activated 1.7 % $(Pd\text{-}EPS)_{aerob}$	50°C, $p(H_2)$=2 MPa, S/C 1000/1, 6 h, THF/water mixture	High activity when activated 1.7 % $(Pd\text{-}EPS)_{aerob}$ was used with very low formation of by-products; possibility to recycle;	Try to reduce the pressure and increase the substrate (**XXXIX**)/molar ratio, try to work using water as unique external solvent
0.28 % Pd/Al_2O_3 *vs* 0.5 % Pd/Al_2O_3	35°C, $p(H_2)$= 0.2 MPa, S/C 2500/1, 5-20 h, toluene	High selectivity toward the C=C double bond under milder condition was observed by using both catalysts. This fact demonstrates the importance of this γ-alumina as support.	Further investigation using different catalysts preparation protocol with or without microwaves
0.28 % Pd/Al_2O_3 *vs* BASF and 0.5 % Pd/Al_2O_3 catalysts	60°C, $p(H_2)$=2 MPa, S/C 32000/1, 2 h, ethanol	Homemade catalyst showed higher activity than BASF catalyst, anyhow in the same condition all catalysts showed less selectivity due to the formation of acetals.	Further investigations on the use of solvent in order to maintain high activity and selectivity
0.28 % Pd/Al_2O_3 *vs* 4.3 % Pd-ENCAT	40°C/50°C, $p(H_2)$=0.5 MPa; S/C 1000/1; 20 h, different solvents	Homemade Pd/Al_2O_3 is more efficient than 4.3 % Pd-ENCATS, which does not appear suitable for this kind of reaction.	
0.18 % Rh/Al_2O_3	50°C; $p(H_2)$ = 5 MPa, S/C 1000/1 and 3800/1, toluene; 60°C; $p(H_2)$ = 2 MPa; S/C m.r 32000/1, ethanol	Respect to 0.18 % Pd/Al_2O_3, no hydrogenation of carbonyl group was detected at 0.5 MPa of H_2 and 50°C. Also in this case, when the reaction was performed under BASF conditions, low selectivity was detected.	Further studies to improve the performance of catalyst.

In conclusion, homemade Pd and Rh based catalysts are very active in the hydrogenation of unsaturated aldehydes. These catalysts work in heterogeneous conditions (0.28 % Pd/Al_2O_3 and 0.18 % Rh/Al_2O_3) or in aqueous biphasic conditions (1.7 % $Pd\text{-}EPS)_{aerob}$ and 1.7 % activated $(Pd\text{-}EPS)_{aerob}$ and these features make these catalytic species very interesting from an environmental and economic point of view and potentially suitable for scaling up.

3.9.Synthesis of Lioral^{TM}: introduction

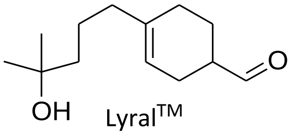
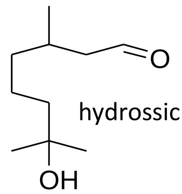
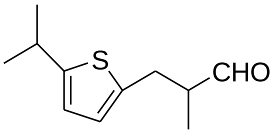
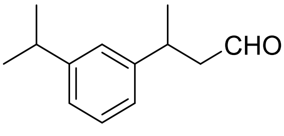
Lioral^{TM} is a fine aldehydic fragrance and presents a floreal (lily of the valley) odour profile. It is an odorant booster, not allergenic and with a good persistence. The synthesis was described only in a patent by Luca Turin[147] (Scheme 3.9.1). However, the description is not exhaustive for what chemical purity and yield are concerned.

Scheme 3.9.1. Synthetic way to obtain Lioral^{TM}

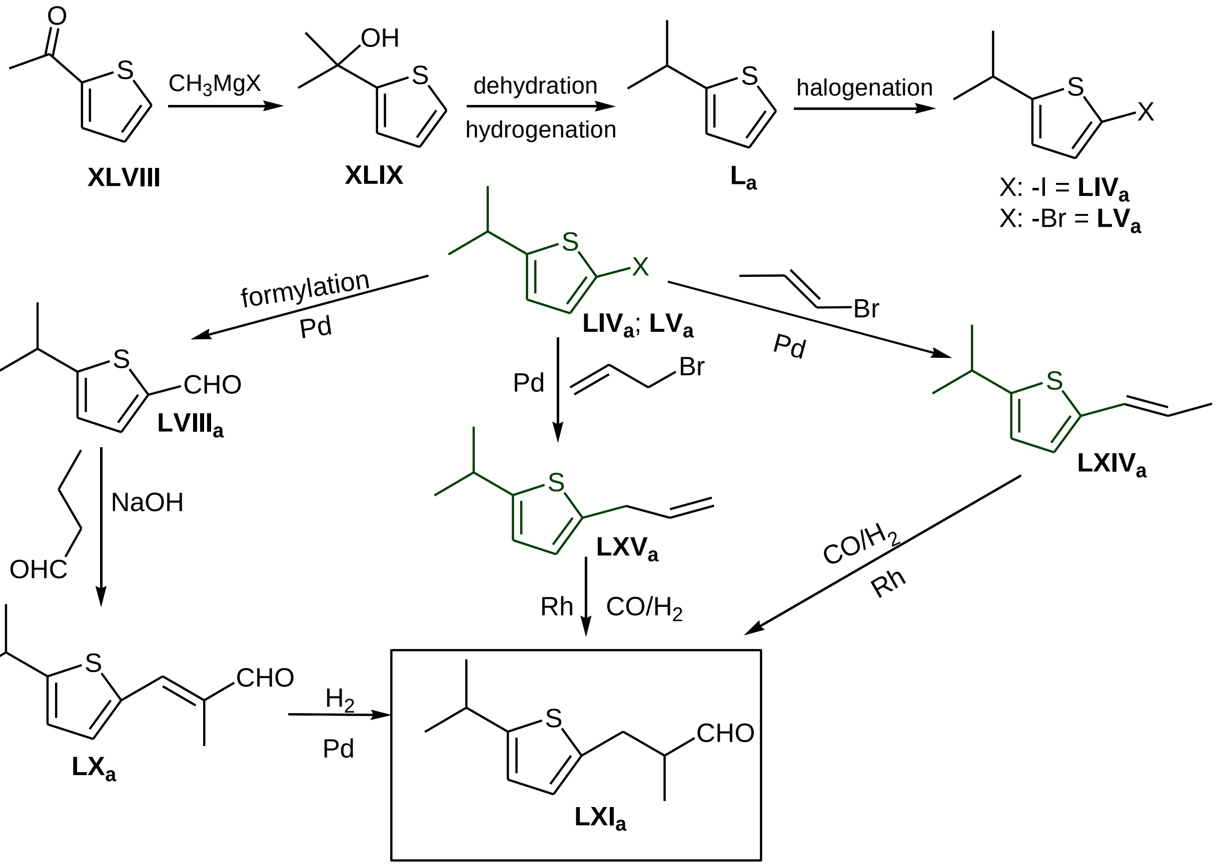


Lioral^{TM} is a high-cost fragrance but has advantages with respect to the competing products, such as for example a higher biodegradability that is relevant to avoid accumulation of the product in the environment. The characteristics of Lioral^{TM} and related products are reported in Table 3.9.1.

Table 3.9.1. Characteristics of some commercial fragrances

Compounds	Characteristics
 Lyral^{TM}  hydrossicitronellal	low persistence; low cost; potentially allergenic; scent of lily of the valley
 Lioral^{TM}	ability to enhance other fragrances; good persistence (no unpleasantness); scent of lily of the valley (but not an aldehyde note); high cost; non-allergenic; properties of the two enantiomers are unknown
 Florhydral^{TM}	scent of lily of the valley; good persistence (slightly unpleasantness in a long period); high cost; different scent and our threshold value of the two enantiomers

Previously Lioral™ was synthesized by Heck reaction or by the use of enzymes to obtain enantiomerically enriched forms[148]. In the present research, new catalysts were evaluated in order to optimize the reaction conditions and to study some alternative ways to obtain Lioral™. In the following Scheme 3.9.2 the various investigated methods are reported.



Scheme 3.9.2. Different studied approaches to obtain Lioral™

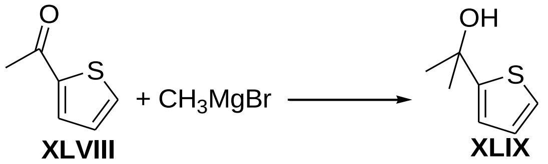
On the basis of fact that many following reaction Scheme 3.9.2 were made on alkyl thiophenes (i.propyl or ethyl), hereafter reagents and products will have the same roman number and distinguished subscript: $_{\mathbf{a}}$) for i.propyl derivatives and $_{\mathbf{b}}$) for ethyl derivatives.

To obtain Lioral™ it is necessary to synthesize 2-isopropylthiophene (**L$_a$**). It is a key precursor and is commercial available only at very high price. For instance, Otava sells it at 7,960 €/Kg and Molport sells it at 301,600 €/Kg. Therefore, different strategies to obtain this key intermediate have been studied and described later. Then, **L$_a$** is halogenated and the halo derivative transformed into the target fragrance following two different reaction pathways. By one of these synthetic routes, the halo-intermediate is converted into the 2-isopropylthiophenecarbaldehyde

(**LVIII$_a$**), that is also a promising potential new fragrance (see Paragraph 3.9.5). This aldehyde **LVIII$_a$** is condensed with propanal under basic conditions and the obtained unsaturated product **LX$_a$** is finally hydrogenated to Lioral^{TM} (**LXI$_a$**). Another possible route, is the synthesis, always starting from the halo-intermediate, the olefins **LXV$_a$** or **LVI$_a$** by Pd catalyzed cross-coupling reaction. Both olefins (**LXV$_a$**, **LVI$_a$**) can be hydroformylated to synthesize Lioral^{TM} (**LXI$_a$**) and its isomers. The yield of the hydroformylation reaction and the distribution of isomeric aldehydes is affected by the different position of C=C double bond in the olefin precursor. Howerer, it would be interesting to evaluate the scent of these mixtures in comparison with pure **LXI$_a$**.

However, it should be underline that because of the high cost and the difficulties to get 2-isopropylthiophene (**L$_a$**) in large amounts, it was decided to study either the optimization of some reaction parameters and new synthetic processes starting from the structural analogue 2-ethyltiophene (**L$_b$**) which is commercially available at lower price (1,652 €/Kg). Furthermore, it would also be interesting to evaluate the potentiality products derived from **L$_b$**. as fragrances and/or flavors.

3.9.1 Synthesis of 2-(thiophen-2-yl)propan-2-ol (XLIX)



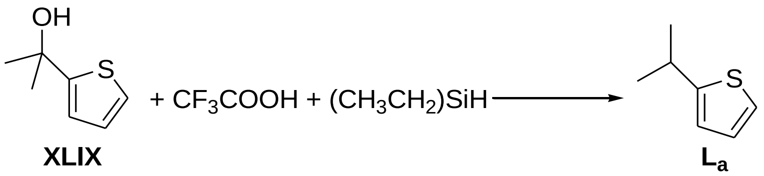
Scheme 3.9.3. Synthesis of 2-(thiophen-2-yl)propan-2-ol (XLIX)

2-(Thiophen-2-yl)propan-2-ol (**XLIX**) is a key intermediate for the preparation of 2-isopropylthiophene (**L$_a$**). The synthesis of **XLIX** was carried out as reported previously[149]. The conversion of **XLVIII** into **XLIX** was quantitative and, after work-up a yellow liquid was obtained with 95 % yield.

3.9.2 Synthesis of 2-isopropylthiophene (L_a) starting from 2-(thiophen-2-yl)propan-2-ol (XLIX)

2-Isopropylthiophene (**L_a**) is the starting material to obtain Lioral™ (**LXI_a**), and different approaches were investigated.

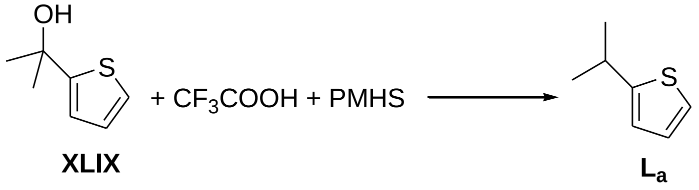
3.9.2.1 Synthesis of 2-isopropylthiophene (L_a) by treatment of 2-(thiophen-2-yl)propan-2-ol (XLIX) with trifluoroacetic acid and triethylsilane (TES)



Scheme 3.9.4. Synthesis of 2-isopropylthiophene (L_a) by using trifluoroacetic acid and TES

In the literature it was reported that it is possible to obtain alkyl substituted thiophenes by reaction of alkenyl thiophenes with trifluoroacetic acid and triethylsilane[150]. In this paper, the authors describe in detail the method to obtain 3-isopropylthiophene and also claim a 90 % yield for the target 2-isomeric compound. For this reason the described protocol was repeated on alcohol **XLIX**. The reaction was fast, but afforded numerous by-products, including a bis(i-propyl-thiophene)dimer. The work-up was not simple and, after distillation, only a 13% yield of the desired product **L_a** was obtained.

3.9.2.2 Synthesis of 2-isopropylthiophene (L_a) by reaction of 2-(thiophen-2-yl)propan-2-ol (XLIX) with trifluoroacetic acid and PMHS [poly(methylhydrosiloxane)]



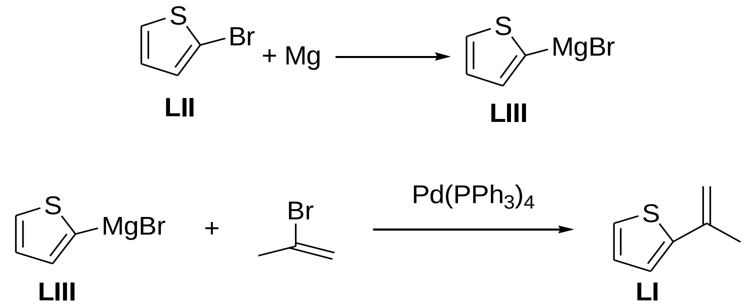
Scheme 3.9.4. Synthesis of L_a by using PMHS and trifluoroacetic acid

At the light of the previous results, PMHS [poly(methylhydrosiloxane)] instead of TES was used as H-donor. In this way, in principle a purer **L_a** could be obtained without traces of volatile silicon by-products. However following the previous procedure, only 13 % conversion into **La** was observed.

3.9.2.3 Synthesis and isolation of 2-(prop-1-en-2-yl)thiophene (LI)

In order to obtain **L_a** another possible approach was the synthesis of 2-(prop-1-en-2-yl)thiophene (**LI**), as intermediate, and its following reduction.

3.9.2.3.1 Synthesis of 2-(prop-1-en-2-yl)thiophene (**LI**) by cross-coupling reaction

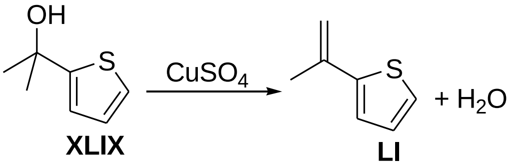


Scheme 3.9.5. Synthesis of 2-(prop-1-en-2-yl)thiophene (LI) by cross-coupling reaction

The synthesis involves the cross-coupling reaction of the Grignard reagent 2-thiophenyl magnesium bromide (**LIII**), easily obtained from the commercially available 2-bromothiophene (**LII**) and Mg turnings, with 2-propenylbromide in the presence of $Pd(PPh_3)_4$[151]: disappointingly, this synthetic route gave unsatisfactory results, being polymeric derivatives the prevailing reaction products.

3.9.2.3.2 Synthesis of 2-(prop-1-en-2-yl)thiophene (**LI**) by dehydration of 2-(thiophen-2-yl)propan-2-ol (**XLIX**) using different dehydrating agents

The synthesis of olefin **LI** was investigated by dehydration of **XLIX** using different agents. First the reaction was carried out by following a procedure described for similar substrates in the presence of copper sulphate as dehydrating agent[152]:



Scheme 3.9.6. Synthesis of LI by dehydrating of XLIX

In order to obtain high yields of the desired product **LI** the dehydration was performed under very mild conditions but only 36 % yield of the isolated product **LI** was recovered

Table 3.9.2. Results obtained by using $CuSO_4$ as dehydrating agent

Run	P (Pa)	Distillation T(°C)	(LI) Isolated Yield (%)
1[a]	----	----	---[d]
2[b]	130	60	29
3[b]	6	25	36

[a] Reaction conditions: substrate **XLIX** = 1 g (0,007 mol); dry $CuSO_4$ = 0,83 g (**XLIX**/cat molar ratio: 1/0,75); T = 25°C; vacuum was not applied; [b] substrate **XLIX** = 2 g (0,014 mol); dry $CuSO_4$ = 1,66 g (**XLIX**/cat molar ratio: 1/0,75); [d] only by-product were obtained.

In order to improve the yield in 2-(prop-1-en-2-yl)thiophene (**LI**), other dehydrating systems were tested and the obtained results are summarized in Table 3.9.3.

Table 3.9.3. Results obtained by using different dehydrating agents

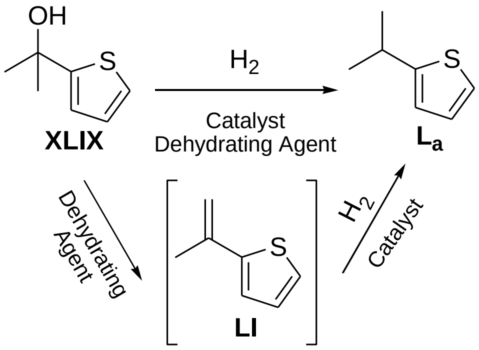
Run	Dehydrating agent	Conv.XLIX (%)	LI (%)	By-product (%)	LI (%) isolated yield
1[a]	Aquivion®	99	---	99	n.d
2[b]	Acidic coal superwj	99	55	45	21
3[c]	CH_3COOH	99	50	50	n.d*

[a] Reaction conditions: substrate **XLIX** = 2 g (0,014 mol); Aquivion® = 0,243 g (sub. **XLIX**/-RSO_3H 50/1); n-Hexane = 10 mL; T = 25°C; t = 2 h; [b] substrate **XLIX** = 5 g (0,035 mol); acidic coal superwj = 1,5 g; n-Hexane = 30 mL; T = 25°C; t = 24 h; [c] substrate **XLIX**= 5 g (0,035 mol); 0,6 g (0,004 mol) of KI, 7 mL of Na_2SO_3 (50 % w/v) water solution; CH_3COOH = 40 mL; n-Hexane= 40 mL; T = 50°C, t = 2 h; * the crude mixture was treated as reported in experimental parts and the olefin **LI** was directly hydrogenated into 2-isopropylthiophene (**L_a**).

Aquivion® is an acidic perfluorinated acid polymer (for further information see Paragraph 3.11) but when it was used as dehydrating agent only solid by-products were formed. This result can be explained by the fact that the sulfonic groups can promote the polymerization of olefin **LI**. Operating with acidic coal or acetic acid (run 2 and 3, Table 3.9.3), compound **LI** was produced but in both cases large amounts of by-products were obtained. After work-up of the crude mixture obtained from run 2, only 21 % of the desired olefin **LI** was recovered. This result is perhaps affected by the difficulty to separate efficiently **LI** from the solvent during the work-up. In run 3, the crude mixture was purified following the work-up described in the experimental parts and then the obtained olefin was hydrogenated into **L_a**, according to the procedure describes in detail in the experimental parts. At the end of the reaction (24 h) conversion, determine by quantitative GC analysis into **L_a** was 80%, but after distillation under vacuum, the target product **L_a** was obtained in only 41 % yield.

3.9.3 Synthesis of 2-isopropylthiophene (L_a) by combined dehydrating/hydrogenation reactions

At the light of obtained results in the previous experiment for the synthesis of **LI**, it appears evident that it is difficult to recover the pure product in high yields. In fact, in the presence of Pd catalyst or acid environment, a high amount of by-products was formed. For these reasons, a different approach was investigated: the dehydration of alcohol **XLIX** was combined with the simultaneous hydrogenation of the intermediate olefin **LI**. By this way, the low-stable **LI** is converted directly into 2-isopropylthiophene $\mathbf{L_a}$ without any intermediate work-up. The scheme of this synthetic pathway is reported below.



Scheme 3.9.7. One step synthesis of L_a by dehydration/hydrogenation

This reaction was deeply studied, using different types of hydrogenation catalysts and different kinds of dehydrating agents. The obtained results are reported in the following Tables 3.9.4, 3.9.5 and 3.9.6.

Table 3.9.4. Results obtained with different dehydrating agents and 5 % Pd/C (Chimet) as hydrogenation catalyst

Run	Dehydrating agent	Conv. XLIX (%)	L_a (%)	By-products (%)
1[a]	Amberlyst 15® (H^+ form)	99	80	19
2[b]	CH_3COOH	99	59	40
3[c]	$CuSO_4$	99	---	99
4[d]	Acidic Coal	99	52	47
5[e]	Acidic Coal	99	28	72*
6[f]	Montmorillonite K-10	72	19	53

General reaction conditions: substrate **XLIX** = 5 g (0,035 mol); Pd/C 5 % 0.53 g (**XLIX**/Pd molar ratio: 140/1); $p(H_2)$ = 0.5 MPa; T = 25°C, t= 24 h; [a] Amberlist-15 (H^+ form) = 0,5 g; BHT = 0,05 g; diisopropylether = 1 mL; isooctane = 30 mL; [b] acetic acid = 1.00 mL (0.018 mol, d=1.05 g/mL); Pd/C 5 % = 0,78 g(**XLIX**/Pd molar ratio: 95/1); BHT = 0.05 g; CH_3OH = 30 mL; [c] dry $CuSO_4$ = 7,5 g (**XLIX**/dry $CuSO_4$ molar ratio 1/0.75); pentane = 30 mL; [d] Acidic coal superwj = 1.5 g; BHT = 0.05 g; diisopropylether = 1 mL; isooctane = 30 mL; T= 30 h; [e] substrate **XLIX** = 3 g (0.021 mol); PMHS = 5 mL; Pd/C 5 % = 0.50 (**XLIX**/cat molar ratio: 92/1); Acid coal superwj = 0.5 g; toluene = 20 mL; T = 25°C; t =72 h; * 33 % is 2-(prop-1-en-2-yl)thiophene (**LI**); [f] substrate **XLIX** = 3 g (0.021 mol); Pd/C 5 % = 0.45 g (**XLIX**/Pd molar ratio: 100/1), pentane = 20 mL; 48 h.

Table 3.9.5. Results obtained by using Pd deposited on different acidic supports

Run	Catalyst	Dehydrating Agent	Conv. XLIX (%)	L_a (%)	By-products (%)
1[a]	Pd/acid coal 1 %	---	99	60	39
2[b]	Pd/acid Al_2O_3 1 %	---	0	0	0
3[c]	Pd/acid Al_2O_3 1 %	---	10	0	10
4[d]	Pd/acid Al_2O_3 1 %	CH_3COOH	80	3	77*

[a] substrate **XLIX** = 5 g (0.035 mol); Pd/acidic coal = 0.5 g; BHT = 0.05 g; diisopropylether = 1 mL; isooctane = 30 mL; $p(H_2)$ = 0.1 MPa; T = 25°C; t = 48 h. [b] substrate **XLIX** = 2 g (0.014 mol); Pd/Al_2O_3 acid 1 % = 1.49 g (sub/Pd molar ratio 100/1); n-Hexane = 20 mL; $p(H_2)$ = 0.5 MPa, T = 25°C, t = 24 h.[c] the same condition of run 2, but the temperature was 50°C and $p(H_2)$ = 0.5 MPa; [d] substrate **XLIX** = 2 g (0.014 mol); Pd/Al_2O_3 acid 1 % = 1.49 g(**XLIX**/Pd molar ratio 100/1); CH_3COOH = 1 mL (0,018 mol, d=1,05 g/mL); n-Hexane = 20 mL; $p(H_2)$= 0.5 MPa; T = 25°C; t = 24 h, * 40 % of 2-(prop-1-en-2-yl)thiophene (**LI**) is included.

Table 3.9.6. Results obtained by using $Pd(OAc)_2$ as hydrogenation catalyst in presence of acetic acid or acetic anhydride as dehydrating agent.

Run	Dehydrating agent	Conv. (XLIX) %	L_a (%)	By-product (%)
1[a]	$(CH_3CO)_2O$	20	2	18
2[b]	CH_3COOH	80	40	40

Reaction conditions: [a] substrate **XLIX** = 2 g (0,014 mol); acetic anhydride = 1 mL (0.009 mol, d=1.09 g/mL); $Pd(AcO)_2$ = 0.032 g (**XLIX**/cat molar ratio: 100/1) activated at $p(H_2)$ = 1 MPa for 1 h before the reaction; n-Hexane = 10 mL; THF = 10 mL; $p(H_2)$= 0.5 MPa; T = 25°C; t = 20 h; [b] substrate **XLIX** = 2 gr (0.014 mol); CH_3COOH = 1 mL (0.018 mol, d=1.05 g/mL); $Pd(AcO)_2$ = 0.032 g (**XLIX**/cat molar ratio: 100/1) activated at $p(H_2)$ = 1 MPa for 1 h; n-Hexane = 10 mL; THF = 10 mL; T = 50°C; $p(H_2)$ = 0.5 MPa; t = 20 h.

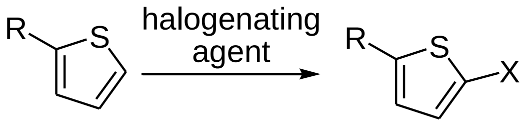
The best result was obtained when the reaction was carried out in the presence of Amberlyst-15® as dehydrating agent and isooctane as solvent (run 1, Table 3.9.3). In this experiment, complete conversion of alcohol **XLIX** was detected and conversion to $\mathbf{L_a}$ was 80 % (determined by calibration line). After work-up, pure desired product $\mathbf{L_a}$ was obtained in 50 % yield.

In general, it was observed that the recovery of 2-isopropylthiophene ($\mathbf{L_a}$) was not simple. It is necessary to have an efficient distillation column because part of 2-isopropylthiophene ($\mathbf{L_a}$) is lost with the distilled solvent. In any case, the combined dehydration/hydrogenation reactions represent a promising synthetic route to obtain 2-isopropylthiophene ($\mathbf{L_a}$) but a careful study for a possible scaling-up of this protocol is necessary. In particular the following points have to be further investigated:

1. the type of hydrogenation catalyst that must be enough active without promoting the polymerization of **LI**;
2. the type of dehydrating agent that must be enough active to dehydrate 2-(thiophen-2-yl)propan-2-ol (**XLIX**), but should be not too strong to increase the formation of by-products (such as $CuSO_4$);
3. the mixing affects the reaction yield so H_2 concentration must be sufficient to promote the hydrogenation reaction without giving time to olefin to polymerize;
4. work-up and purification procedure must be optimized even if, as alternative, in order to reduce $\mathbf{L_a}$ losses, it should be possible to use directly the crude mixture in the following reaction steps.

3.9.4 Halogenation of 2-alkylthiophenes

The halogenation reaction was studied either on 2-isopropylthiophene ($\mathbf{L_a}$) or on 2-ethylthiophene ($\mathbf{L_b}$) by using different halogenating systems (Scheme 3.9.8).

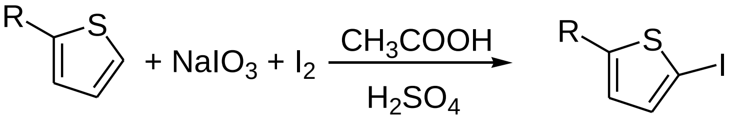


R: -Et = $\mathbf{L_b}$
R: -iPr = $\mathbf{L_a}$

R: -Et; X: I = $\mathbf{LIV_b}$
R: -Et; X: Br = $\mathbf{LV_b}$
R: -Et; X: Cl = $\mathbf{LVI_b}$
R: -iPr; X: I = $\mathbf{LIV_a}$
R: -iPr; X: Br = $\mathbf{LV_a}$

Scheme 3.9.8. Halogenation of alkylthiophenes

3.9.4.1 Iodination of 2-alkylthiophenes



R: -Et = $\mathbf{L_b}$
R: -iPr = $\mathbf{L_a}$

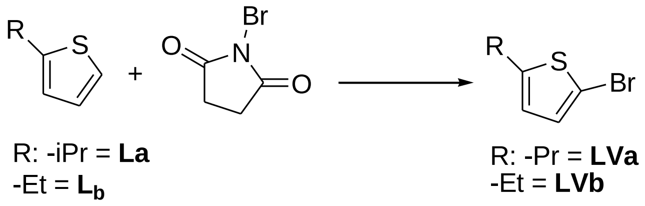
R: -Et = $\mathbf{LIV_b}$
R: -iPr = $\mathbf{LIV_a}$

Scheme 3.9.9. Iodination of 2-alkylthiophenes

The iodination was performed by using the $NaIO_3/I_2$ redox system in ethyl acetate as the solvent[153]. This synthesis was carried out on 2-ethyl $\mathbf{L_b}$ and 2-isopropyl thiophene ($\mathbf{L_a}$); in both cases conversion was quantitative and selectivity almost complete, being other mono halo-isomer formed in negligible amount (< 1%). Moreover, di-halogenated compounds were never detected in the reaction mixture.

3.9.4.2 Bromination of 2-alkylthiophenes

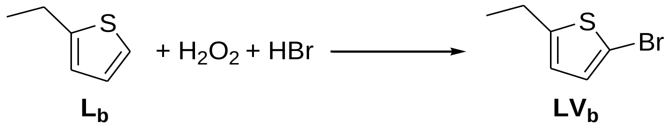
The bromination of 2-alkylthiophenes of $\mathbf{L_a}$ and $\mathbf{L_b}$ was performed by using different systems. Initially, N-bromosuccinimide (NBS) was used as brominating agent[154]:



Scheme 3.9.10. Bromination of 2-alkylthiophenes by using N-bromosuccinimide

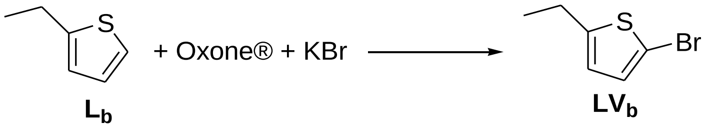
The reaction was carried out at 25°C, in a mixture 1:1 of acetic acid and chloroform for two hours. Conversion was very high (> 99 %), but the formation of β-isomer (about 2 %) was detected. The presence of β-isomer in the reaction mixture, even if in small amounts, is a relevant drawback as it is very difficult to remove it by distillation or by flash chromatography. Analogously to the iodination, also in this case di-halogenated compounds were not formed.

Besides the formation of β-isomer, it should be emphasized the use of chloroform as solvent and N-bromosuccinimide as brominating agent. The first one is toxic for human health and it is not eco-friendly while N-bromosuccinimide leads to the formation of N-succinimide as byproduct. Therefore, to avoid all these drawbacks, the reaction was carried out by using HBr/H_2O_2 system[155]:



Scheme 3.9.11 Bromination of 2-ethyllthiophenes (L_b) by using HBr/H_2O_2 system

This system uses HBr as brominating agent and a 1:1 mixture of CH_3CN and water as solvent. Acetonitrile is not exactly eco-friendly but is necessary to use as co-solvent because 2-alkylthiophenes are not very soluble in water. Conversion to 2-bromo-5-ethylthiophene (**LV_b**) was 87% and after 5 h also 4 % of di-brominated compounds and traces of isomeric monobromo derivative was formed. Another investigated brominating system was KBr/Oxone®[156]:



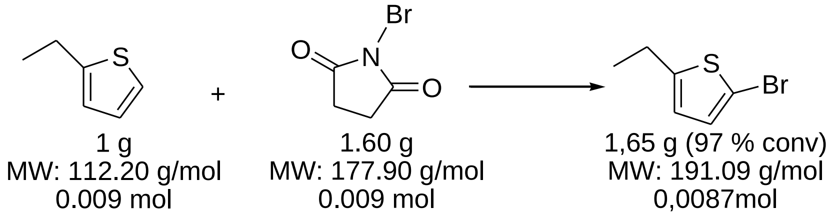
Scheme 3.9.12. Bromination of 2-ethyllthiophene (LV_b) by using KBr/Oxone® system

Oxone®, also note as Caroat or MPS is a triple salt composed by potassium mono persulfate with the following formula: $KHSO_5 \bullet KHSO_4 \bullet K_2SO_4$ and the molecular weight is 614.76 g/mol (corresponding to 152.2 g/mol for $KHSO_5$). The reaction was carried out at 25°C in a 1:1 mixture of CH_3CN and water as solvent, and after 2 h 90 % conversion of substrate was detected. 82 % of (**LV_b**), 8 % of di-brominated compounds and traces of isomeric monobromo derivative compound were found.

3.9.4.2.1 Analysis of bromination reaction

The bromination, as above described, was carried out by using three different systems. Here, the comparison of the methods used, according to some green chemistry parameters (green metrics), are reported. The following calculations are based on reactions with 1 g of **L_b** as starting material.

a) Reaction with N-bromosuccinimmide:

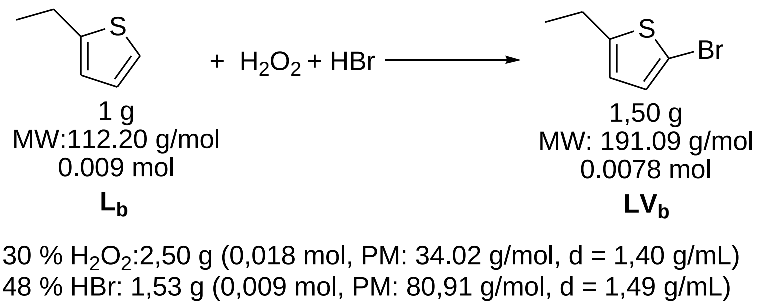


L_b

LV_b

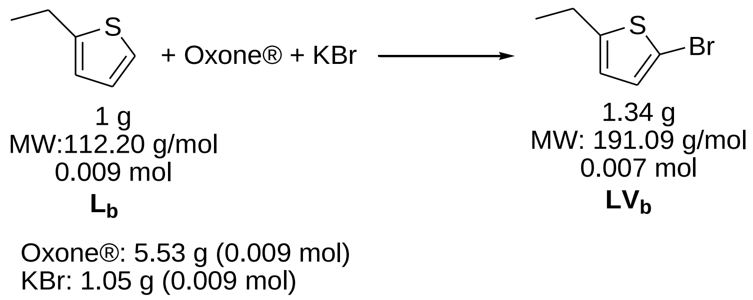
Scheme 3.9.13. Bromination of (L_b) by using N-bromosuccinimmide

b) Reaction with H_2O_2/HBr system



Scheme 3.9.14. Bromination of L_b by using HBr/H_2O_2

c) Reaction with Oxone®/KBr system:



Scheme 3.9.15. Bromination of L_b by using KBr/Oxone®

Table 3.9.7. Comparison between the various reactions of bromination according to some green metrics

Brominating agent	Conv. L_b (%)	LV_b (%)	By-products (%)	A.E	RME	E
N-bromosuccinimmide	99	97	2	0.66	0.63	8.22
H_2O_2/HBr system	91	87	4	0.85	0.64	8.65
Oxone®/KBr	90	82	8	0.23	0.18	19.01

It is important to remember that a “green reaction” is characterized by AE and RME value close to one, and E value close to zero. For definitions and further considerations on these parameters see Table 1.3, paragraph 1.2.

From obtained results, it is possible to observe that, from environmental point of view, as the preliminary conclusion, the best method to obtain bromo-derivatives is the reaction carried out by using the brominating system H_2O_2/HBr despite the not complete conversion. The reaction was carried out on mixture of water and acetonitrile and the detected conversion of starting material **L_b** was 91 %, with high selectivity, in fact, 87 % of desired product **LV_b** was found. 4 % of di-brominating by-product was also found but they are easily separable from desired **LV_b** by distillation. Furthermore, is important to note that the by-products obtained from this brominating system are essentially dirty water and oxygen[155].

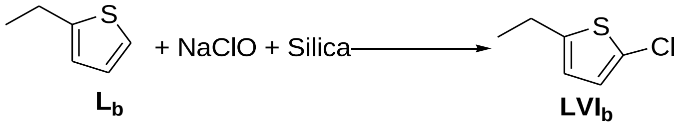
This last point is important for "environmental" economy. In fact, when N-bromosuccinimide was used as brominating agent, high conversion and selectivity was found (97 % conversion of $\mathbf{L_b}$ into $\mathbf{LV_b}$) and comparable value of AE, RME and E-factor was determined. However, the reaction was carried out in chloroform and acetic acid that represent the major waste at the end of reaction. Furthermore, produced N-succinimide as residual of the brominating system is found in the wastes (it represents 45 % in weight of brominating system) and should be recovered and reuse to regenerate NBS.

The green parameters calculated for the reaction carried out by using Oxone® and KBr are affected by the molecular weight of the salt and from lower selectivity of this brominating system. In fact, despite the high conversion of $\mathbf{L_b}$ (90 %) only 82 % represents $\mathbf{LV_b}$ inasmuch 8 % of di-brominated by-product was also detected. The reaction was carried out in mixture of water and acetonitrile and the wastes are mainly represented from dirty water, unreacted Oxone® and its derivative salt, KOH and unreacted KBr. In this last case, it is possible to improve the selectivity of reaction by calibrating the amount of reagents and studying in detail the best mode to introduce them; however the residue salt from Oxone® still represent a major drawback in AE, RME and E factor parameters.

This exercise of evaluation of the sustainability a type of reaction, using simple green metrics, could not be so precise and, as already in Chapter 1, for industrial study, many other parameters including LCA approach are mandatory.

3.9.4.3 Chlorination of 2-ethylthiophene (LVI$_b$)

The chlorination of **L$_b$** was carried out by using a solution of HClO buffered at pH = 8, in presence of silica:



Scheme 3.9.16. Chlorination of L$_b$ by using NaClO and Silica

The reaction was carried out as reported in experimental parts, and after 21 h, conversion to 2-chloro-5-ethylthiophene (**LVI$_b$**) was 90 %. Besides the desired product, traces of 5-ethylthiophen-2-ol (**LVII$_b$**) or other hydroxylthiophene isomers were detected in the reaction mixture. This result is very interesting and cheap. It is to underline that the previous references in the literature this reagents combination are scanty[157,158] and never applied in thiophene derivatives.

3.9.5. Synthesis of aldehydes by reductive carbonylation

As depicted in Scheme 3.9.2, halo-derivatives of **L$_a$** and **L$_b$** were transformed corresponding carboxaldehyde (**LVIII$_a$** and **LVIII$_b$**), a valuable intermediate for the synthesis of the fragrance Lioral^{TM}(**LXI$_a$**) and analogous compounds.

Aldehydes can be prepared by using different methodologies[159] but carbonylation can be considered one of the most interesting synthetic way, also from an industrial point of view. As a matter of fact, the synthesis of aldehydes by carbonylation reaction presents many advantages with respect to the classical methods that are affected by the use of large amounts of reagents, by the presence of multiple steps and, at least, by the production of a stoichiometric amount of wastes (Table 3.9.8).

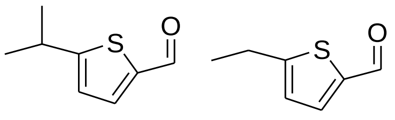
Table 3.9.8. Reactions to obtain aldehydes

Substrate	Reagents	Name reaction
Ar-H	HCN/HCl/Lewis Acids	Gattermann
Ar-H	CO/HCl/Lewis Acids	Gattermann-Koch
Ar-H	RCN/HCl/Lewis Acids	Hoesch
Ar-H	$POCl_3$/N,N-dimethylaniline	Vilsmeyer
Ar-CH_2X	Urotropine/H_2O	Sommelet
Ar-X	Triethyl orthofomate/H_2SO_4/H_2O	Chichibabin
Ar-X	CO/H-donor/catalyst	Heck

However, the conditions initially reported by Heck were not suitable for industrial applications, since they involved high pressure (greater than 8 MPa of 1/1 CO/H_2) and high amount of catalyst[160]. Over the past 30 years, many studies have been done to improve the reaction conditions. For example Stille and co-workers useed metal hydrides as reducing agents; in this way different substrates were successfully carbonylated in 2.5–3.5 h under mild conditions (50-80°C, 0.1–0.3 MPa CO) by using tributyltin hydride (Bu_3SnH)[161]. However, tin hydrides could not be used today because of their toxicity and waste generation. Other reducing agents have been applied such as PMHS (polymethylhydrosiloxane)[162] and other organ-silanes[163,164] in the presence of various known palladium catalytic systems. From these work, it is clear that an accurate setting of the reaction parameters (catalyst, base, solvent, temperature, pressure, concentration) for the transformation of (hetero)aryl bromides and iodides into the corresponding aldehydes is fundamental. Generally, the desired aldehydes were obtained in 79-100 % yield[164].

It is also important to note that the use of carbon monoxide is not as desirable because of its toxicity and gaseous nature. Consequently, different CO sources have been investigated such as formate salts or, recently, the use of paraformaldehyde[165]. However, despite the research on hydrogen and CO alternatives, homogeneous metal based catalysts modified with phosphino ligands are still used and could hamper its industrial application.

The interest of carbonylation in this work of thesis was focused on the synthesis of two thiophene carboxaldehyde derivatives (**LVIII$_a$** and **LVIII$_b$**):

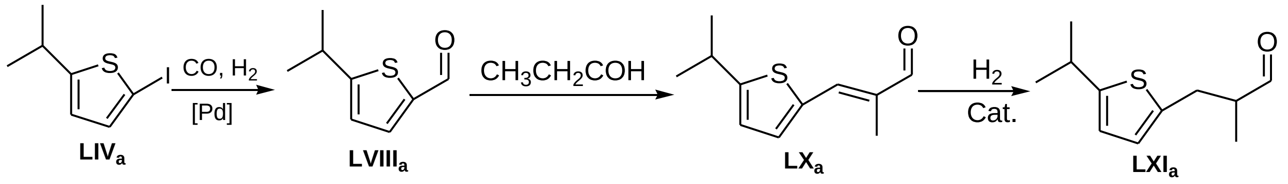


LVIII$_a$ **LVIII$_b$**

Figure 3.9.1. Aldehydes synthesized by reductive carbonylation

5-Ethylthiophen-2-carbaldehyde (**LVIII$_b$**) is an industrial flavor of commercial interest and can be formed in natural way during foods cooking. Some studies have shown that this aldehyde is retrievable in cooked beef and cooked pork liver[166]. It is one of the substances responsible for the typical aroma and smell of these foods and it is formed by Maillard reaction which occurs during the foods cooking[167].

Compound **LVIII$_a$** is a fragrance with a potential industrial interest[168] but here is also important intermediate to synthesize Lioral™ (**LXI$_a$**):



Scheme 3.9.17. Synthetic route to obtain Lioral™ (LXI$_a$)

3.9.5.1 Result of Carbonylation reaction

In the literature it is reported that aldehydes can be obtained from aryl-halides by using triethylsilane (TES)[163] or polymethylhydrosiloxane (PMHS) as H-donor[162]. Basing on these data, initially the study was focused on the synthesis of **LVIII$_a$**. The reaction of carbonylation was carried out, as reported in the literature, starting from 5-iodo-2-isopropylthiophene (**LIV$_a$**) and by using PMHS as H-donor in the presence of $Pd(PPh_3)_4$ (run 1) as catalyst and hexamethylphosphoroamide (HMPA) as base . The solvent was acetonitrile[162].

Table 3.9.8. Results obtained by carbonylation of LIV_a under homogeneous conditions

Run	Base	Solvent	Conv. LIV_a (%)	$LVIII_a$ (%)	L_a (%)
1[a]	HMPA	CH_3CN	99	97	2
2[b]	Na_2CO_3	DMF	99	69	30
3[c]	Na_2CO_3	Me-THF	99	99	---

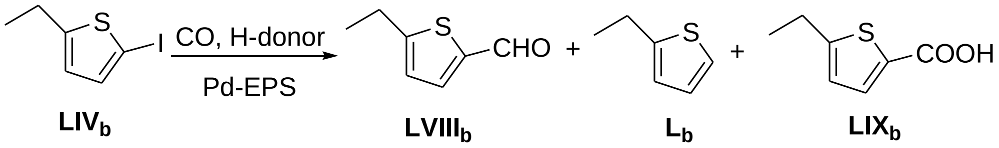
[a]Reaction conditions: substrate **LIV_a** = 2 g (0.008 mol); PMHS = 3.36 mL; HMPA = 5.57 mL (HMPA/**LIV_a** molar ratio 4/1 d = 1.03 g/mL); $Pd(PPh_3)_4$ = 0.092 g (**LIV_a**/cat molar ratio 100/1); CH_3CN = 10 mL; p(CO) = 1 MPa; T = 80°C; t = 20 h; [b] same conditions of test 1 but 1.70 g of Na_2CO_3 (rap Na_2CO_3/**LIV_a** molar ratio 2/1) was used instead of HMPA, and 10 mL of DMF was used instead of CH_3CN; [c]same condition of test 1 but 1.70 g of Na_2CO_3 (rap Na_2CO_3/**LIV_a** molar ratio 2/1) was used instead of HMPA, 0.051 g of $Pd(PPh_3)_4$ with **LIV_a**/cat molar ratio 180/1 was used and 10 mL of 2-MeTHF was used instead of CH_3CN.

Even if the result of the reaction according to run 1 (Table 3.9.8) is excellent, hexamethylphosphoroamide (HMPA) is not a safe compound. It has been shown to cause nasal cancers in rats, and many organic chemists regard HMPA as an exceptionally hazardous molecule due to its known carcinogenicity [169]. Also the use of DMF (run 2, Table 3.9.8) is not advisable, as this solvent can irritate noise and throat, may damage the developing fetus and seems that it can cause spontaneous abortions and miscarriages in exposed woman[170].

For these reasons, in this work, HMPA was replaced with sodium carbonate while CH_3CN and DMF were replaced with a greener solvent, 2-MeTHF. The reaction was carried out at 1 MPa of CO pressure and 80°C for 20 h, by using a substrate **LIV_a**/Pd molar ratio 180/1: conversion was almost complete (99%) and aldehyde **$LVIII_a$** was the only reaction product. Noteworthy, at these reaction conditions and by using safer reagents respect to those used in runs 1 and 2, substrate dehalogenation did not occur, being **L_a** never detected in the reaction mixture. After distillation, pure 5-isopropylthiophen-2-carbaldheyde (**$LVIII_a$**) was recovered as transparent liquid.

However, this process had the drawback of using the homogeneous catalyst $Pd(PPh_3)_4$ so its recovery was practically impossible. To overcome this problem, the study of the reaction by using easily recoverable and recyclable catalysts such as heterogeneous ones or water soluble catalytic systems operating in an aqueous biphasic environment was carried out.

As concerning to biphasic aqueous systems, the interest was focused on the biogenerated catalyst activated 1.7 % $(Pd\text{-}EPS)_{aerob}$. In a first approach, the carbonylation reaction on 2-iodo-5-ethylthiophene (**LIV_b**) was studied.



Scheme 3.9.18. Carbonylation of LIV$_b$

Initially the reaction was carried out with 3.5 MPa of H_2 and 3.5 MPa of CO at 80°C for 24 h, with sodium carbonate as base and with a substrate **LIV$_b$**/Pd molar ratio = 50/1, only 2-ethylthiophene (**L$_b$**), deriving from the substrate dahalogenation, was formed (run 1). Also operating with a lower amount of catalyst (**LIV$_b$**/Pd molar ratio = 100/1) and lower pressure (3 MPa of CO and 20 MPa of H_2) the desired aldehyde **LVIII$_b$** was not obtained; however, it was found the formation of 5-ethylthiophene-2-carboxylic acid (**LIX$_b$**) (22% yield). The acid formation is probably due to the competitive reaction with water. Working in the absence of hydrogen, a quantitative conversion of 2-iodo-5-ethylthiophene (**LIV$_b$**) into 5-ethylthiophene-2-carboxylic acid (**LIX$_b$**) was found. Another test, always in the presence of syngas, was carried out using quinine as base. Operating in this way, a further conversion decrease was detected, but besides 2-ethylthiophene (**L$_b$**) (27 %) and 5-ethylthiophene-2-carboxylic acid (**LIX$_b$**) (18 %) the formation of the desired aldehyde (**LVIII$_b$**) (23 %) was observed (run 3, Table 3.9.9).

Table 3.9.9. Carbonylation of LIV$_b$ catalyzed by activated 1.7% (Pd-EPS)$_{aerob}$

Run	p(CO) MPa	p(H_2) MPa	Base	Conv. LIV$_b$ (%)	LVIII$_b$ (%)	L$_b$ (%)	LIX$_b$ (%)
1[a]	3.5	3.5	Na_2CO_3	99	-	99	-
2[b]	3	2	Na_2CO_3	85	-	63	22
3[b,c]	3	2	Quinine	68	23	27	18
4[b]	5	-	Na_2CO_3	99	-	-	99

[a] Reaction conditions: substrate **LIV$_b$** = 0.024 g (0,1 mmol); activated Pd-EPS 1.7 % = 0.010 g (**LIV$_b$**/Pd molar ratio = 50/1; Na_2CO_3 = 0.021 g (base/**LIV$_b$** molar ratio 2/1); H_2O = 2 mL; THF = 2 mL; T = 80°C; t = 24 h. [b] same conditions of test 1 but the **LIV$_b$**/cat molar ratio is 100/1 (0.005 g of activated Pd-EPS) and the base/**LIV$_b$** molar ratio was 2/1 (0.005 g of Na_2CO_3). [c]same condition of test 2, but 0.016 g of quinine (base/**LIV$_b$** molar ratio 2/1) was used instead of Na_2CO_3.

In order to improve the aldehyde yield, some carbonylations were carried out by using H-donors instead of hydrogen and the results are reported in Table 3.9.10.

Table 3.9.10. Carbonylation of (LIV$_b$) catalyzed by activated (1.7% Pd-EPS)$_{aerob}$ by using H-donor (PMHS and TES)

Run	T (°C)	p(CO) MPa	Base	Conv. LIV$_b$ (%)	LVIII$_b$ (%)	L$_b$ (%)	LIX$_b$ (%)
1[a]	80	5	Na_2CO_3	84	31	53	-
2[b]	80	5	Na_2CO_3	93	68	5	20
3[c]	80	5	Na_2CO_3	99	88	3	8
4[c,d]	80	5	Na_2CO_3	99	82	-	17
5[c,d]	80	5	Na_2CO_3	98	85	Traces	13
6[c,d]	80	5	Na_2CO_3	55	42	13	---
7[e]	80	5	Quinine	99	93	2	5
8[e,f]	80	5	Quinine	99	81	9	9
9[e,f]	80	5	Quinine	61	16	22	23
10[g]	80	5	Na_2CO_3	97	76	17	4
11[g,h]	80	5	Na_2CO_3	99	85	6	8
12[g,h]	80	5	Na_2CO_3	98	67	23	8
13[g,h]	80	5	Na_2CO_3	22	21	1	Traces
14[i]	60	5	Na_2CO_3	89	82	7	Traces
15[i,j]	60	5	Na_2CO_3	77	64	Traces	13
16[k]	60	5	Quinine	67	67	Traces	---
17[k,l]	60	5	Quinine	45	43	2	---
18[m]	60	1	Na_2CO_3	75	69	6	---
19[n]	60	1	Quinine	50	48	2	---
20[o]	60	5	TMEDA	92	75	17	---

[a] Reaction conditions: substrate **LIV$_b$** = 0.024 g (0,1 mmol); PMHS = 0.042 mL; activated Pd-EPS 1.7 % = 0.005 g (**LIV$_b$**/Pd molar ratio = 100/1); Na_2CO_3 = 0.021 g (base/**LIV$_b$** molar ratio 2/1); H_2O = 2 mL; THF = 2 mL; p(CO) = 5 Mpa, T = 80°C; t = 24 h; [b] 0.116 g (0.1 mmol) of TES as H-donor was used; [c] same condition of run 1, but the **LIV$_b$**/base molar ratio is 2/1 (0.005 g of Na_2CO_3) and H-donor was 0.116 g (0.1 mmol) of TES; [c,d] recycling of run 3; [e] same conditions of run 1, but H-donor is 0.116 g (0.1 mmol) of TES and Quinine is used as base (**LIV$_b$**/base molar ratio 2/1; 0.016 g); [e,f] recycling of run 7; [g] substrate **LIV$_b$** = 0.115 g (0.5 mmol); TES = 0.58 g (0.5 mmol); activated Pd-EPS = 0,005 g (Sub (**LIV$_b$**)/Pd molar ratio = 500/1); Na_2CO_3 = 0.027 g (base/**LIV$_b$** molar ratio 1/2); H_2O = 2 mL; THF = 2 mL; p(CO) = 5 MPa; T = 80°C; t = 24 h; [g,h] recycling of run 10; [I] substrate **LIV$_b$** = 0.024 g (0,1 mmol); TES = 0.116 g (0.1 mmol); activated Pd-EPS = 0.005 g (**LIV$_b$**/Pd molar ratio = 100/1), Na_2CO_3 = 0.005 g (base/**LIV$_b$** molar ratio 1/2); H_2O = 2 mL; THF = 2 mL; p(CO) = 5 MPa, T = 60°C; t = 24 h; [i,j] recycling of run 14; [k] substrate **LIV$_b$** = 0.024 g (0,1 mmol); TES = 0.116 g (0.1 mmol); activated Pd-EPS = 0.005 g (**LIV$_b$**/Pd molar ratio = 100/1); quinine = 0.016 g (base/**LIV$_b$** molar ratio 1/2); H_2O = 2 mL; THF = 2 mL; p(CO) = 5 MPa; T = 60°C; t = 24 h; [k,l] recycling of run 16; [m] same conditions of run 14, but the p(CO) = 1 MPa; [n] same conditions of run 16 but the p (CO)= 1 MPa; [o] same conditions of run 14, but TMEDA as base was used (0.003 g, base/**LIV$_b$** molar ratio 1/2, d = 0.78 g/mL.

When PMHS was used (run 1, Table 3.9.10), a high substrate **LIV$_b$** conversion was observed (84 %), however the main product was **L$_b$**. So, in order to improve the reaction selectivity, TES was tested to better control of hydride amount.

The reaction was carried out at the conditions reported in Table 3.9.10; 93 % conversion was observed, but only 68 % **LVIII$_b$** formed (run 2); moreover, a relevant formation of acid **LIX$_b$** (20 %) was detected. Reducing the quantity of catalyst (**LIV$_b$**/cat molar ratio 500/1) and using an equivalent amount of base respect to substrate **LIV$_b$**, it was possible obtain an almost complete substrate **LIV$_b$** conversion (99%) with 88 % of formation of the desired aldehyde **LVIII$_b$** (run 3). In this case the aqueous catalytic phase was recovered and recycled. Fresh Na_2CO_3 was added for each recycling and high activity was observed for the first and second recycling experiment (runs 4 and 5) but in third test an activity loss was detected. This fact could be explained by supposing accidental losses of the catalyst, even if in very small extents, during the recycles of the catalytic phase, by poisoning due to the presence of an excess of base or by a possible partial oxidation of palladium. Using quinine rather than Na_2CO_3 in equivalent amount with respect to **Lb**, it was observed a 99 % conversion and an increase of selectivity towards **LVIII$_b$** (93 %) (run 7, Table 3.9.10). Also in this case, due to the high activity of activated 1.7 % $(Pd\text{-}EPS)_{aerob}$, the catalyst was recycled. In the first recycling experiment the activity of the catalyst was maintained but the selectivity was a little decreased (run 8, Table 3.9.10); when the catalyst was recycled for the second time conversion was only 61 % with 16 % of the desired aldehyde **LVIII$_b$**.

At the light of the high activity of this catalyst, the substrate **L$_b$**/catalyst molar ratio was increase up to 500/1: operating under the conditions reported in Table 3.9.10 a high conversion of 2-ethyl-5-iodothiophene (**LIV$_b$**) was obtained but the formation of the desired aldehyde **LVIII$_b$** decreased (run 10, table 3.9.10). However, it was possible to recycle the catalyst three times and only in the third recycling experiment a decrease of activity was detected (run 12, Table 3.9.10).

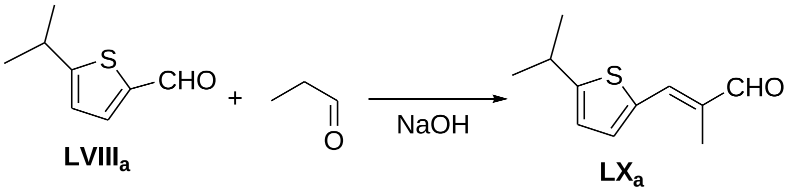
In order to improve the conversion into the desired product **LVIII$_b$**, the reaction was also carried out at 60°C, 5 MPa of CO using TES as H-donor and quinine or sodium carbonate as base (runs 13 and 16, Table 3.9.10). In both experiments, the substrate conversion decreased but the amount of 5-ethylthiophen-2-carbaldheyde (**LVIII$_b$**) was enhanced (runs 14 and 16, Table 3.9.10). Unfortunately, the catalytic activity progressively decreased with the catalyst recycling (runs 15 and 17, Table 3.9.10). When tetramethylethylenediamine (TMEDA) was used as base instead of quinine, working always at 60°C and 5 MPa of CO, a higher conversion was detected but the selectivity toward aldehyde **LVIII$_b$** decreased, being **L$_b$** formed with 17% yield.

The carbonylation of 2-iodo-5-ethylthiophene (**LIV$_b$**) was also carried with homemade catalyst 0.28 % Pd/Al_2O_3 in the absence of any phosphine. Preliminary results, obtained by working at the conditions reported in experimental part, were very promising; in fact, 68 % of substrate conversion and complete selectivity toward the desired aldehyde **LVIII$_b$** were obtained.

Carbonylation was also studied by using bromo **LV$_b$** or chloro **LVI$_b$** derivatives as substrate in order to improve the economy of the process. Following the procedure reported in the literature[171], complete conversion of substrate **LV$_b$** and high selectivity toward the desired aldehyde **LVIII$_b$** (87 %) was found. It is important to remark that, in this case, homogeneous conditions were used by using $Pd(OAc)_2$, DABP (diadamanthylbutylphosphine) as ligand and TMEDA as base. Furthermore, it was necessary to adopt a substrate/catalyst molar ratio 4/1 instead of 40/1 as reported in literature for similar compounds. As a matter of fact, when 40/1 molar ratio was used, reaction did not occur. This result can be considered a good starting point for the development of carbonylation on cheaper substrates such as bromo-derivatives.

Unfortunately, when the reaction was carried out on 2-chloro compound **LVI$_b$** with different catalysts system and variety of reaction conditions, no desired target was found.

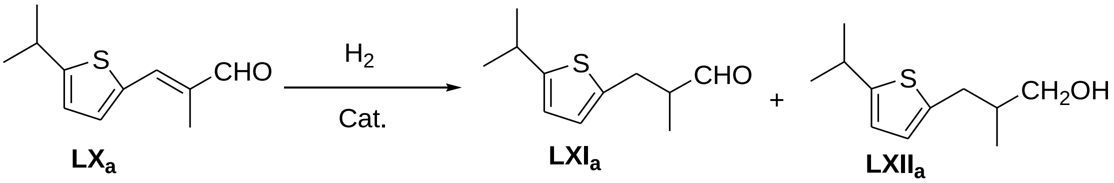
3.9.6 Synthesis of 3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (LX$_a$)



Scheme 3.9.19. Synthesis of LX$_a$

The reaction was performed at 60°C for 3 h, and conversion into desired compounds **LX$_a$** was 80 %; after work-up yield was 65%.

3.9.7 Hydrogenation of 3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (LX_a) catalyzed by Pd-EPS species



Scheme 3.9.20 Hydrogenation of 3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (LX_a)

The hydrogenation reaction was carried out in the presence of (1.7% Pd-EPS)$_{aerob}$ and the results are reported in Table 3.9.11.

Table 3.9.11. Hydrogenation of 3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (LX_a) catalyzed by 1.7% Pd-EPS

Run	Conv. LX_a (%)	LXI_a (%)	$LXII_a$ (%)
1	53	53	---
2[a]	25	25	---
3[b]	73	72	1
4[a,b]	39	38	1

Substrate **LX_a** = 0.100 g (1.96 mmoli); 1.7 % Pd-EPS = 0.012 g (**LX_a**/Pd molar ratio 1000/1); H_2O = 4 mL; THF = 4 mL; p(H_2) = 4 MPa; T = 50°C; t = 22h.

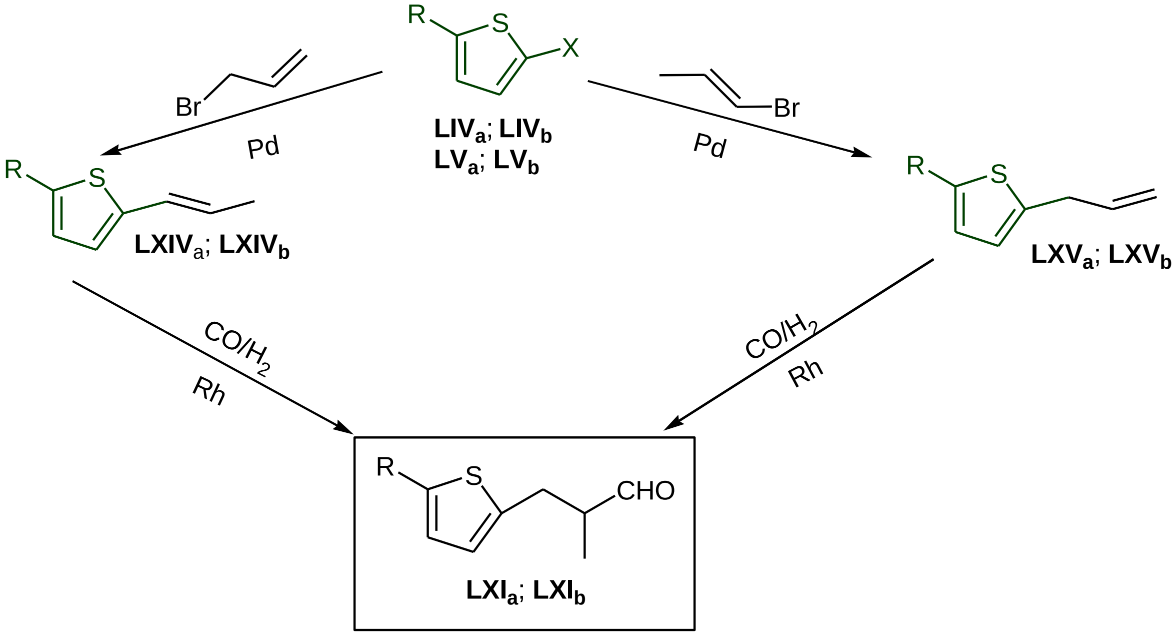
[a]: experiment carried out by using the catalytic phase recovered from the previous run.

[b]: **LX_a**/Pd = 500/1 (molar ratio)

Working at 50°C and 4 MPa of H_2 for 22h conversion was not very high but selectivity to the desired saturated aldehyde **LXI_a** was complete using a **LX_a**/Pd molar ratio 500/1 (runs 3 and 4) or 1000/1 (runs 1 and 2, Table 3.9.11). These results demonstrated that the catalyst is very high selective toward the carbon-carbon double bond hydrogenation; however, it was observed a deactivation of the catalyst in recycling experiments. As a matter of fact, when the reaction was carried out by using the catalyst recovered from runs 1 and 3, respectively, conversion was strongly decreased but selectivity remained very high (runs 2 and 4, Table 3.9.11).

3.9.8 Synthesis of olefin intermediates: 2-isopropyl-5-(prop-1-enyl)thiophene (**LXIV$_a$**), 2-ethyl-5-(prop-1-enyl)thiophene (**LXIV$_b$**), 2-allyl-5-isopropylthiophene (**LX$_a$**) and 2-allyl-5-ethylthiophene (**LXV$_b$**) by cross-coupling reaction

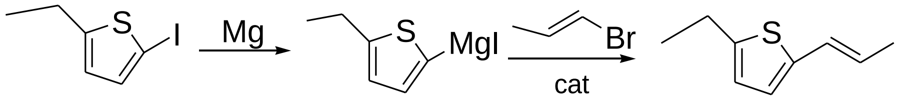
Another potential way to obtain Lioral™ (**LXI$_a$**) and "pseudoLioral" (**LXI$_b$**), where alkyl group is ethyl instead of i.propyl, is represented by the hydroformylation of suitable olefin precursors (Scheme 3.9.21):



Scheme 3.9.21 Other possible synthetic pathway to obtain Lioral™ (LXI$_a$) and similar structure compounds

However, at the moment, only **LXIV$_b$** and **LXV$_b$** were synthetized, in order to obtain a suitable model, in larger amount, to study the following hydroformylation reaction with homemade Rh-based catalysts. The analogous derivatives, where i.propyl group was present, had been already prepared in smaller scale[148].

Compounds **LXIV$_b$** and **LXV$_b$** were obtained by adopting the procedure reported in the literature for similar substrates[151]:

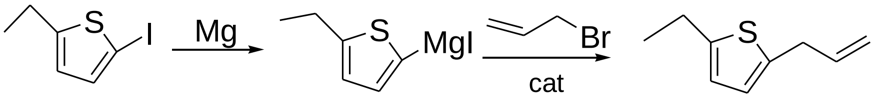


LIV$_b$ **LXIII$_b$** **LXIV$_b$**

Scheme 3.9.22. Synthesis of 2-ethyl-5-(prop-1-enyl)thiophene (LXIV$_b$)

The reaction between Grignard reagent and 1-bromoprop-1-ene [(E)/(Z) mixture] was carried out in dry diethyl ether at reflux temperature using $Pd(PPh_3)_4$ as catalyst. After 2 hours conversion was complete: 92 % of the desired olefin **LXIV$_b$** [(E)/(Z) mixture], 2 % of 2-ethylthiophene (**L$_b$**) and 6 % of high boiling point by-products were found in the reaction mixture. After work-up and distillation at 1 Pa and 85°C, **LXIV$_b$** was obtained in 63 % yield.

The same procedure was applied to obtain 2-allyl-5-ethylthiophene **LXV$_b$**, always using $Pd(PPh_3)_4$ as catalyst:



LIV$_b$ **LXIII$_b$** **LXV$_b$**

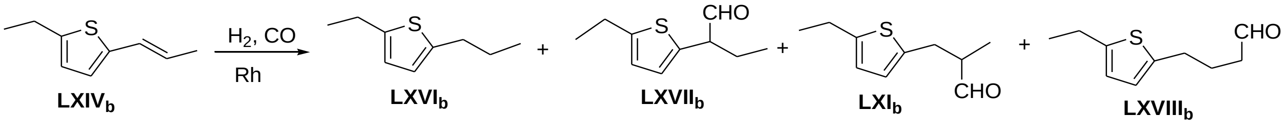
Scheme 3.9.23. Synthesis of 2-allyl-5-ethylthiophene (LXV$_b$)

The reaction gave good results: conversion was 90 % and the yield of the desired olefin **LXV$_b$** was 70 %; disappointingly also 20 % of by-products were formed including of **LXV$_b$** position isomer (3-8 %). After work-up, the desired product **LXV$_b$** was obtained by distillation at 1 Pa and 89°C (yield 65 %).

3.9.9 Synthesis of 3-(5-ethylthiophen-2-il)-2-methylpropanal (LXVI$_b$) (and isomeric aldehydes)

The hydroformylation reaction was investigated using the homemade Rh catalysts (1.2% Rh-EPS and 0.18% Rh/Al_2O_3) and, for comparison, with two well-known rhodium catalysts ($HRh(CO)(PPh_3)_3$ and $Rh(CO)_2(acac)$/TPPTS) on both isomeric olefins (**LXIV$_b$** and **LXV$_b$**) to verify how the position of the double bond could affect the yield of the desired product and the isomeric distribution.

Initially the reaction was carried out by using olefin **LXIV$_b$**.



Scheme 3.9.24. Hydroformylation of LXIV$_b$

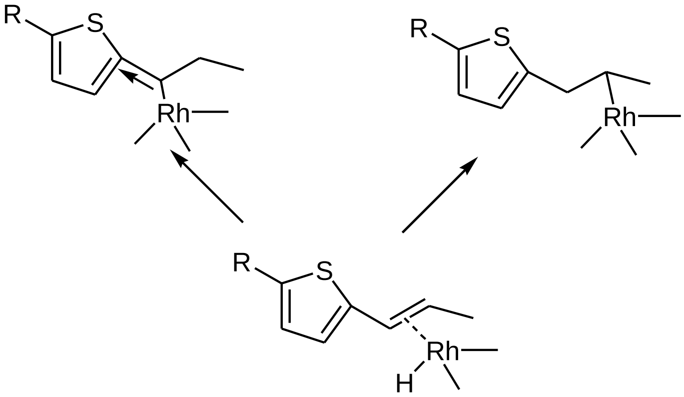
Table 3.9.12. Hydroformylation LXIV$_b$ the presence of rhodium catalysts

Run	Cat.	Sub/cat molar ratio	Conv. LXIV$_b$ (%)	LXVI$_b$ (%)	LXVII$_b$ (%)	LXI$_b$ (%)	LXVIII$_b$ (%)
1[b]	Rh-EPS 1.2%	500	49	6	21	22	Traces
2[b,c]	Rh-EPS 1.2 %	500	45	3	20	19	3
3[b,c]	Rh-EPS 1.2 %	500	14	1	6	6	1
4[a]	Rh-EPS 1.2 %	100	60	6	22	28	4
5[d]	Rh/Al_2O_3 0.18 %	500	---	---	---	---	---
6[e]	Rh/Al_2O_3 0.18 %	100	79	6	47	26	---
7[f]	$HRh(CO)(PPh_3)_3$	1000	94	6	71	17	---
8[g]	$Rh(CO)_2(acac)$/TPPTS	1000	27	1	15	11	---

Reaction condition: [a] substrate **LXIV$_b$** = 0.110 g (0.73 mmol); 1.2 % Rh-EPS = 0.063 g; H_2O = 2 mL; toluene = 2 mL; $p(CO)=p(H_2)=3$ MPa, T = 60°C, t = 20 h; [b] substrate **LXIV$_b$** = 0.11 g (0.73 mmol); 1.2 % Rh-EPS = 0.013 g; H_2O = 2 mL; toluene = 2 mL; $p(CO)=p(H_2)=4$ MPa, T = 100°C, t = 20 h; [c] run carried out with recovered catalyst from run 3; [d] substrate **LXIV$_b$** = 0.013 g (0.087 mmol); 0.18 % Rh/Al_2O_3 = 0.01 g; toluene = 10 mL; $p(CO)=p(H_2)=4$ MPa; T = 80°C, t = 20 h; [e] substrate **LXIV$_b$** = 0.013 g (0.087 mmol); 0.18 % Rh/Al_2O_3 = 0.05 g; toluene = 10 mL; $p(CO)=p(H_2)=4$ MPa; T = 80°C, t = 20 h; [f] substrate **LXIV$_b$** = 0.195 g (1.3 mmol); $HRh(CO)(PPh_3)_3$ = 1.2 mg; toluene = 2 mL; $p(CO)=p(H_2)=3.5$ MPa; T = 80°C, t = 24 h; [g] substrate **LXIV$_b$** = 0.195 g (1.3 mmol); $HRh(CO)(PPh_3)_3$ = 1.2 mg; TPPTS = 4.4 mg (cat/TPPTS molar ratio 1/6); toluene = 2 mL; H_2O = 2 mL; $p(CO)=p(H_2)=3.5$ MPa; T = 80°C; t = 24 h.

The reaction was carried out at 8 MPa of syngas and 100°C for 20h, by using 1.2% Rh-EPS with a substrate **LXIV$_b$**/Rh molar ratio 500/1: conversion was 49 % but the yield of pseudo-Lioral **LXI$_b$** was only 22%, being an equimolar mixture of aldehydes **LXI$_b$** and **LXVII$_b$** formed. Moreover, also 3% of the terminal aldehyde **LXVIII$_b$** was found (run 1, Table 3.9.12), probably due to the capability of the catalyst to isomerize the internal double bond to the terminal position.

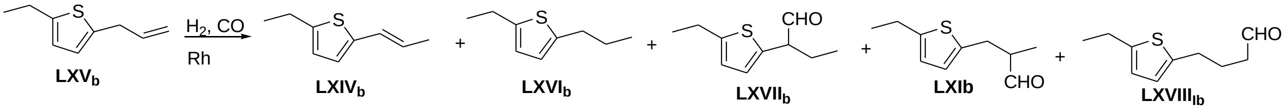
The aqueous catalytic phase maintained its activity in the first recycling experiment (run 2, Table 3.9.12) but when the catalyst was used in a second recycle the activity strongly decreased (run 3, Table 3.9.12). It is possible to suppose that sulfur, close to the C=C double bond, can strongly interact with rhodium and, as a consequence, partially deactivate the catalyst. In order to increase the conversion a substrate **LXIV$_b$**/Rh molar ratio 100/1 was used but no more than 60% conversion was obtained and the yield of pseudoLioral **LXI$_b$** was only 28% (run 4, Table 3.9.12). When 0.18 % Rh/Al_2O_3 was used, it was observed that working with a 500/1 substrate **LXIV$_b$**/catalyst molar ratio the reaction did not occur (run 5, Table 3.9.12). Operating at the same conditions but reducing the substrate **LXIV$_b$**/catalyst molar ratio to 100/1, 79 % conversion was obtained (run 6, Table 3.9.12). In this case, a large amount of aldehyde **LXVII$_b$** was formed, probably due to the lower steric hindrance of the catalytic system with respect to 1.2 % Rh-EPS. Working with homogeneous catalyst as Wilkinson hydride (run 7, Table 3.9.12) it was possible to carry out the reaction with a substrate **LXIV$_b$**/molar ratio 1000/1 at 80°C and 7 MPa of syngas. Conversion was very high (94%) but the regioselectivity towards pseudoLioral **LXI$_b$** was very low. In fact the main product was α-aldehyde **LXVII$_b$** and the obtained ratio between α and β aldehyde is approximately 4. This fact could be explained by the favorite attack on the α-carbon respect to thiophene ring that allows to obtain an intermediate stabilized by resonance (Scheme 3.9.25):



Scheme 3.9.25

The reaction was carried out also in an aqueous biphasic system in the presence of $Rh(CO)_2(acac)$ modified with the water soluble phosphine TPPTS. This catalyst showed a low activity (27% conversion) and, analogously to what observed with 1.2 % Rh-EPS, the ratio between α-aldehyde (**LXVII$_b$**) and β-aldehyde (**LXI$_b$**) was almost 1:1 (run 8, Table 3.9.12).

The hydroformylation was also studied on olefin **LXV$_b$** and the results are reported in the follow Table 3.9.13.



Scheme 3.9.26. Hydroformylation of LXV$_b$ in the presence of rhodium catalysts

Table 3.9.13. Results obtained by hydroformylation of LXV$_b$

Run	Cat.	Conv. LXV$_b$ (%)	LXIV$_b$ (%)	LXVI$_b$ (%)	LXVII$_b$ (%)	LXI$_b$ (%)	LXVIII$_b$ (%)
1[a]	Rh-EPS 1.2 %	99	15	---	3	35	46
2[a,b]	Rh-EPS 1.2 %	99	10	---	2	40	47
3[a,b]	Rh-EPS 1.2 %	99	17	---	---	36	46
4[a,b]	Rh-EPS 1.2 %	99	20	---	1	33	45
5[a,b]	Rh-EPS 1.2 %	99	21	---	1	32	45
6[c]	Rh/Al_2O_3 0.18 %	99	5	1	3	44	46
7[d]	$HRh(CO)(PPh_3)_3$	99	4	---	---	42	53
8[e]	$Rh(CO)_2$(acac)/TPPTS	94	6	6	6	35	41
9[e,f]	$Rh(CO)_2$(acac)/TPPTS	61	8	8	1	20	24

Reaction condition: [a] substrate **LXV$_b$** = 0.110 g (0.73 mmol); 1.2 % Rh-EPS = 0.013 g (**LXV$_b$**/cat molar ratio 500/1); toluene = 2 mL; H_2O = 2 mL; p(CO)=p(H_2)=4 MPa; T = 100°C, t = 20 h; [b] run carried out with recovered catalyst from run 1; [c] substrate (**LXV$_b$**) = 0.013 g (0.087 mmol); 0.18 % Rh/Al_2O_3 = 0.01 g (**LXV$_b$**/cat molar ratio 500/1); toluene = 10 mL; p(CO)=p(H_2)=4 MPa, T = 100°C, t = 20 h; [d] substrate **LXV$_b$** = 0.195 g (1.3 mmol); $HRh(CO)(PPh_3)_3$ = 1.2 mg(**LXV$_b$**/cat molar ratio 1000/1), p(CO)=p(H_2)=3.5 MPa; TPPTS = 4.4 mg (cat/TPPTS molar ratio 1/6); toluene = 2 mL; H_2O = 2 mL; p(CO)=p(H_2)=3.5 MPa, T = 80°C, t = 24 h, [f] run carried out with recover catalyst from run 8.

1.2% Rh-EPS confirmed to be active in the hydroformylation reaction and its activity was maintained in four recycling experiments (run 1-5, Table 3.9.13). As it was to be expected the formation of the terminal aldehyde was favored due to the lower steric hindrance of the terminal position of the double bond. Very similar activity was shown also by 0.18 % Rh/Al_2O_3 and a lower amount of hydrogenated product was formed (run 6, Table 3.9.13). The use of both $HRh(CO)(PPh_3)_3$ and the water soluble $Rh(CO)_2$(acac)/TPPTS did not improve the selectivity towards the desired pseudoLioral **LXI$_b$** being the terminal aldehyde C the prevailing reaction product (run 7-9, Table 3.9.13).

In conclusion, the use of the heterogeneous catalyst as Rh/Al_2O_3 seems to be a good alternative to the use of homogeneous catalysts. In fact the results obtained in the hydroformylation of the terminal olefin **LXV$_b$** are competitive with those obtained by using homogeneous catalysts. The substrate **LXV$_b$**/cat molar ratio is higher than when Wilkinson hydride was used, but the heterogeneous catalyst is easy to recover and to recycle. For this reason, in the future, it will be important to study the activity of this catalyst in recycling experiments. As regarding to Rh-EPS, the best results were obtained when the terminal olefin was used. In this case, the catalyst was recycled four times and the conversion in all recycling tests was quantitative. Also the selectivity remained practically unchanged during all tests. This is probably explained by the high stability of the catalyst which has no tendency to migrate into the organic phase.

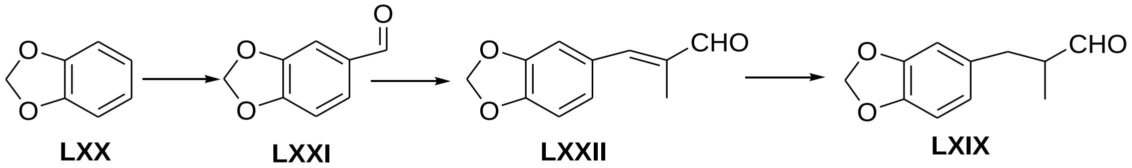
3.10 Synthesis of Helional^{TM} (LXIX)

Helional^{TM} (**LXIX**) is a fragrance used in both feminine and masculine perfumes, shampoos, soaps etc. and presents a green, floral (cyclamen) odor profile with top notes of marine fresh, ozone and new mown hay.

This fragrance is sold at 18-20 $/Kg, and the worldwide production is 400t/year. The main manufacturers are IFF Inc., Givaudan SA, Ube Inc., Takasago Inc., Agan LTD. It is sold as a racemic product even though it was found that the (S)-enantiomer has a threshold of perceptibility much lower of the (R)-enantiomer (0.64 ng / L vs 3.43 ng / L)[172].

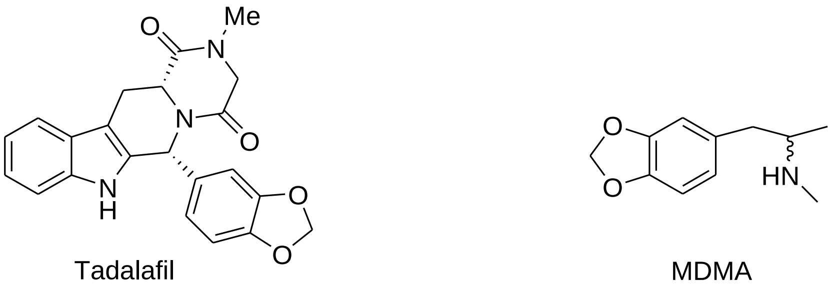
The (S)-enantiomer presents a floral aroma, with a hint of freshness from sea water and ozone, particularly appreciated, as well as the sweet taste of caraway, while the (R)-enantiomer presents the notes of lily of the valley, lemon and an aldehydic character[172].

Starting from benzo[d][1,3]dioxole (**LXX**), it is possible to obtain the desired product **LXIX** by the following synthetic scheme:



Scheme 3.10.1 Synthesis of Helional^{TM} (LXIX)

It is worth noting that in addition to the desired product **LXIX**, another important molecule as benzo[d][1,3]dioxole-5-carbaldehyde (**LXXI**), better known as piperonal or heliotropine, is obtained. It has a floral odor commonly described as being similar to that of vanillin and cherry and for this reason it is used as flavoring. However, it is also an important intermediate for the synthesis of some medicines and drugs.



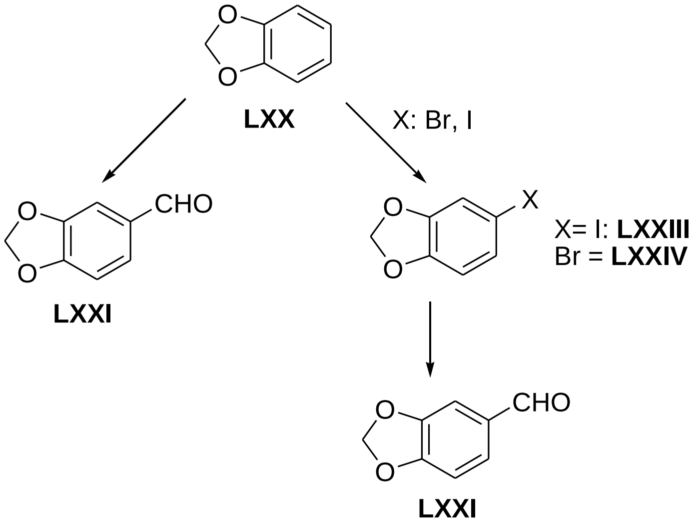
Tadalafil

MDMA

Figure 3.10.1. Piperonal (LXXI) is an intermediate for the synthesis of Tadalifil™ and MDMA

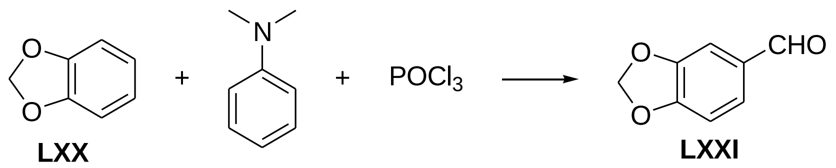
3.10.1 Synthesis of piperonal (LXXI)

The synthesis of piperonal (**LXXI**) was carried out by using two different approaches. In the first one, **LXXI** was synthetized by Vilsmeier reaction, while in the second one, **LXX** was initially halogenated and subsequently carbonylated (Scheme 3.10.2):



Scheme 3.10.2 Different pathways to synthesize Piperonal (LXXI)

3.10.1.1 Synthesis of piperonal (LXXI) by Vilsmeier reaction



Scheme 3.10.3 Synthesis of piperonal (LXXI) by Vilsmeier reaction

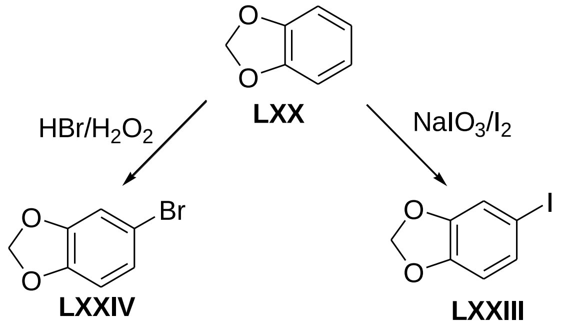
The synthesis of piperonal (**LXXI**) was performed by adopting the procedure described in literature for similar compound[173].The reaction was carried out between **LXX** and N,N-dimethylformanilide in $POCl_3$ at 90°C but after 24 h only 32 % conversion into the desired product **LXXI** was obtained.

3.10.1.2 Synthesis of piperonal (LXXI) by carbonylation reaction

At the light of the good results previously obtained in the carbonylation of hetero-aromatic halo-compounds, it was decided to synthesize **LXXI** by reductive carbonylation of 2-I- or 2-Br-benzodioxole (**LXXIII** and **LXXIV**).

Benzo[d][1,3]dioxole (**LXX**) was brominated or iodinated by adopting the procedure described in the experimental part.

The bromination was carried out by using the HBr/H_2O_2 brominating system. After 24 h, conversion into the desired compound **LXXIV** was 90%, and after distillation under vacuum the pure product was obtained with 80 % yield. Iodination was carried out by using $NaIO_3/I_2$ system, and after 3 h conversion was 92 % into desired compound **LXXIII**. After distillation under vacuum, the pure product **LXXIII** was obtained in 81 % yield.

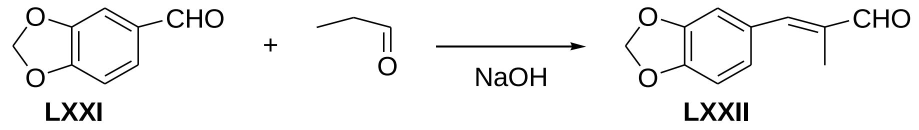


Scheme 3.10.4 Halogenation of Benzo[d][1,3]dioxole (LXX)

The halo derivatives were carbonylated in the presence of homogeneous catalysts. The bromo-derivative **LXXIV** was converted into **LXXI** by $Pd(OAc)_2$/diadamantil butyl phosphine with a substrate **LXXIV**/catalyst molar ratio 4/1 at 1 MPa of syngas (CO/H_2=1) and 100°C. After 24 h, completely conversion into **LXXI** was found. 5-Iodobenzodioxole (**LXXIII**) was carbonylated by using $Pd(PPh_3)_4$ with a substrate **LXXIII**/catalyst molar ratio 180/1 by adopting the procedure reported in experimental part. After 24 h at 1 MPa of CO and 80°C in presence of PMHS as H-donor 66 % conversion in **LXXI** was detected and after distillation under vacuum, the pure desired compound **LXXI** was obtained in 53 % yield.

3.10.2 Synthesis of (E)-3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (LXXII)

The synthesis of (E)-3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (**LXXII**) was performed by adopting the procedure reported in experimental part (paragraph 5.15.8.6):

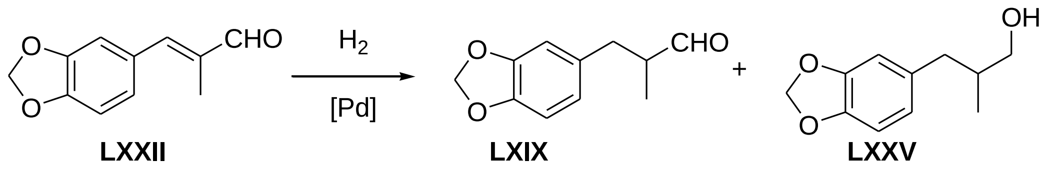


Scheme 3.10.5 Synthesis of (E)-3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (LXXII)

The reaction between **LXXI** and propanal was carried out at 60°C for 3 h. The conversion into the desired compound **LXXII** was 71 % and after purification by flash chromatography (n-hexane/diethyl ether 8/2), **LXXII** was obtained in 58 % yield.

3.10.3 Hydrogenation of (E)-3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (LXXII)

(E)-3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (**LXXII**) was hydrogenated in the presence of homemade palladium catalysts to obtain Helional™ (**LXIX**):



Scheme 3.10.6 Hydrogenation of (LXXII)

The unsaturated substrate **LXXII** was initially hydrogenated in an aqueous biphasic system by using 1.7 % Pd-EPS or activated 1.7 % Pd-EPS as catalyst at 50°C and 2 MPa of H_2:

Table 3.10.1. Hydrogenation of LXXII catalyzed by 1.7 % Pd-EPS and activated 1.7 % Pd-EPS

Run	Cat	$p(H_2)$ MPa	Conv. (LXXII) (%)	LXIX (%)	LXXV (%)
1[a]	1.7 % Pd-EPS	2	52	52	-
2	Recycle	2	32	32	-
3[a]	1.7 % Pd-EPS	4	83	83	-
4	Recycle	4	54	54	-
5[a]	Activated 1.7 % Pd-EPS	2	81	81	-
6	Recycle	2	61	61	-

[a]Reaction conditions: substrate **LXXII** = 0.372 g (0,00196 mol); 1.7 % Pd-EPS = 0.012 g (substrate **LXXII**/catalyst molar ratio 1000/1); THF = 4 mL; H_2O = 4 mL,; T = 50°C; t = 22h.

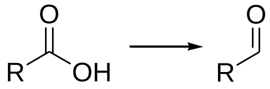
Both 1.7 % Pd-EPS that activated 1.7 % Pd-EPS showed to be very selective towards C=C double bonds, however, conversion was not high (run 1 table 3.10.1) and it was necessary to increase hydrogen pressure from 2 MPa to 4 MPa to obtain about 80% conversion (run 3 and 5).

Disappointingly, both catalysts showed a decreased activity in recycling experiments (run 2, 4, 6, table 3.10.1): these results were rather surprising especially with respect to the results obtained in the hydrogenation of the structurally correlated cinnamaldehyde (paragraph 3.8). It is possible to hypothesize or a poisoning of the catalyst or an accidental loss of the catalytic system, even if in a very small amount, during the separation of phase.

As the polysaccharidic matrix, in which palladium is embedded, presents some chiral centers, the possibility to obtain enantiomerically enriched Helional^{TM} (**LXIX**) were investigated. The mixture recovered from the experiment carried out by using 1.7 % $(\text{Pd-EPS})_{aerob}$ and activatated-1.7 % $(\text{Pd-EPS})_{aerob}$ was treated by $NaBH_4$ to reduce the aldehyde to the corresponding alcohol. Than the mixture was analysed by chiral HPLC equipped with a CHIRACEL OD (250 x 10 µm 4.6 mm) column and using a n-hexane/propanol (97/3) mixture as mobile phase. Unfortunately, in both cases, a racemic mixture of **LXXV** was found. It is interesting to note that in literature[174] the direct analysis of aldehyde **LXIX** under the same conditions is described. However, when the standard racemic mixture of **LXIX** was analyzed, no separation was detected. For this reason, the reduction of aldehyde **LXIX** into alcohol **LXXV** was made.

3.11. Reduction of a carboxylic group to an aldehyde: an enzymatic approach

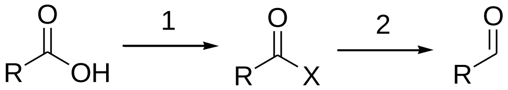
As already reported, there are various methods to obtain aldehydes (hydroformylation, carbonylation) and one of them is the reduction of carboxylic acid that they are widely available substrate and they are precursor to obtain chemicals for the pharmaceutical, agricultural and food industries:

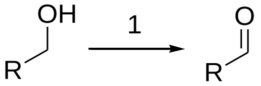


Scheme 3.11.1. Reduction of generic carboxylic acid into aldehyde

However, the direct synthesis is particularly difficult because this pathway could involve toxic or dangerous reagents and drastic conditions. For instance, an aldehyde can be directly obtained by acid reduction by working with lithium in ethylamine or using $LiAlH_4$ and DIBALH at very low temperature, but generally the yield may be low because the aldehyde may be easily reduced to the corresponding alcohol during this reaction[159]. For this reason, in order to obtain high selectivity and yield, to reduce the acids to alcohols and oxidize them to aldehydes or to transform the carboxylic acid into a derivative then it is treated in different manner, is preferred such as summarized in the following Table 3.11.1[159].

Table 3.11.1: Different methods to obtain aldehydes from acid derivatives or alcohols

Synthesis of aldehydes from carboxylic acid derivates			
			
1	**X**	**2**	**Reaction**
$-SOCl_2$	-Cl	Pd on $BaSO_4/H_2$	Rosenmund
$-SOCl_2$	-Cl	$LiAlH(OtBu)_3$	
	$-NR_2$	$LiAlH_4$	
$-SOCl_2$	-Cl	a)$PhNH_2$/b)PCl_5/c)$SnCl_2$	Sonn-Müller*
	-OR	a)N_2H_4/b)$PhSO_2Cl$/c)Na_2CO_3	McFayden and Stevens*
	-CN	a)HCl/b)$SnCl_2$-HCl/c)H_2O	Stephen*

Synthesis of aldehydes from alcohol		
		
R	**1**	**Name of reaction**
Primary aliphatic alcohol	$K_2Cr_2O_7-H_2SO_4$	
	Cl_2	
	$(CO)_2Cl_2$/DMSO	Swern
	Dicyclohexylcarbodiimide	
	O_2/Ag	
	$-H_2$/Ag	
	NaClO-KBr/TEMPO; I_2/TEMPO	
Benzyl alcohol	N_2O_4	Field
	Zeolite	Oppenauer

[*] The methods involves multiple step and various intermediates obtained by transformation of RCOX.

As can be seen these methods are tedious and sometimes involve many steps with different derivatives (e.g. Sonn-Müller, McFayden and Stevens, Stephen reactions) and sometimes involved unsafety reagent such as derivatives of Cr, Cl_2 etc and drastic conditions. Furthermore a lot of wastes are produced. It is clear that finding a technology that allows to obtain selectively aldehydes from acids in a simple way and without the use of special reagents could have positive environmental repercussions and be economically advantageous. Among the various possibilities, a biocatalysed synthesis would be advantageous for various reasons such as the possibility to perform the reaction in aqueous medium under mild conditions obtaining high selectivity to the desired aldehyde. Recently, the use of carboxyreductase enzymes is reported as high selective method to obtain aldehydes from carboxylic acid[37,175,176,177] under milder conditions. However it is also important to note that these enzymes are not commercially available and expensive NADPH cofactor is used as H-donor and should be recycled. Nowadays, these two facts are limiting the spread of this technology of producing aldehydes that could allow environmental benefits.

Furthermore, it must be considered that in certain industrial sectors such as for example the production of aromas and fragrances, the synthesis of a product starting from natural source and using bio-catalysis rather than chemo-catalysis lead to target labeled as natural, but in a cheaper way then the corresponding product obtained by extraction, and highly preferred by the consumer as reported in the Table 3.11.2[178].

Table 3.11.2. Comparison between products obtained by extraction natural, bio-catalysis and chemo-catalysis[178]

Features	Natural flavors		Similar natural flavors/synthetic flavors	
	Extraction	Bio-catalysis	Bio-catalysis	Chemo-catalysis
Origin of the raw material	Natural	Natural	Synthetic	Synthetic
Availability of raw material	Poor	Medium-high	High	High
Stereo selectivity	----	High	High	Medium
Environmental compatibility	Medium-high	High	High	Medium
Price	Very high	High	Medium-high	Low
Consumer preference	Very high	High	Medium	Poor

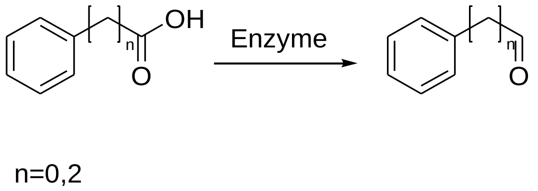
3.11.1 Use of Carboxyreductase (CAR)

The characteristics of enzymes such as substrate selectivity and often very high activity and TOF are well known, but the possibility to work under mild conditions, in water and without unsafe reagents is equally important. Venkitasubramanian et al.[176,177] reported that CAR from Nocardia sp. NRRL 5646 catalyzed the reductive reaction of carboxylic acids. Good results for reduction of many aromatic and aliphatic acids into the corresponding aldehydes were found, in particular benzoic acid, ferulic acids and their analogous.

These authors also observed that the activity of the enzyme depends on the origin of the used microorganism. In fact, for example, in this work it is reported that the relative activity of CAR from Nocardia sp. NRRL 5646, Nocardia asteroids JCM 3016 and Neurospora crassa is different. Suitable substrates for this reaction here described However, the major problem to study this reaction is that the carboxyreductase (CAR) enzymes are not commercial available. For this reason, during this work of thesis a preliminary investigation for the preparation and the application of a CAR enzyme was began, using Mycobacterium marinium as microorganism.

This type of CAR was very recently described in literature[37] and in this work the possibility to combine CAR enzyme and other enzymes such as aldehyde decarbonylase and aldehyde reductase to obtain respectively fatty alkane or fatty alcohol from fatty acid is also described. Only aliphatic acids were tested in this work.

Due to the interest of the research group where this thesis work was made for some aldehydic flavors and fragrances containing an aromatic or heteroaromatic ring, the possibility to reduce some carboxylic acids containing a phenyl ring into the corresponding aldehydes, using CAR enzyme, was verified:



Scheme 3.11.2. Structure of the examined substrates

In particular, 3-phenylpropanal (**VI**), which has a green, aldehydic, floral and melon odor, could be an interesting future industrial target, qualified as natural fragrance, if this biocatalyzed synthetic pathway would be applied to a natural 3-phenylpropanoic acid (**XL**).

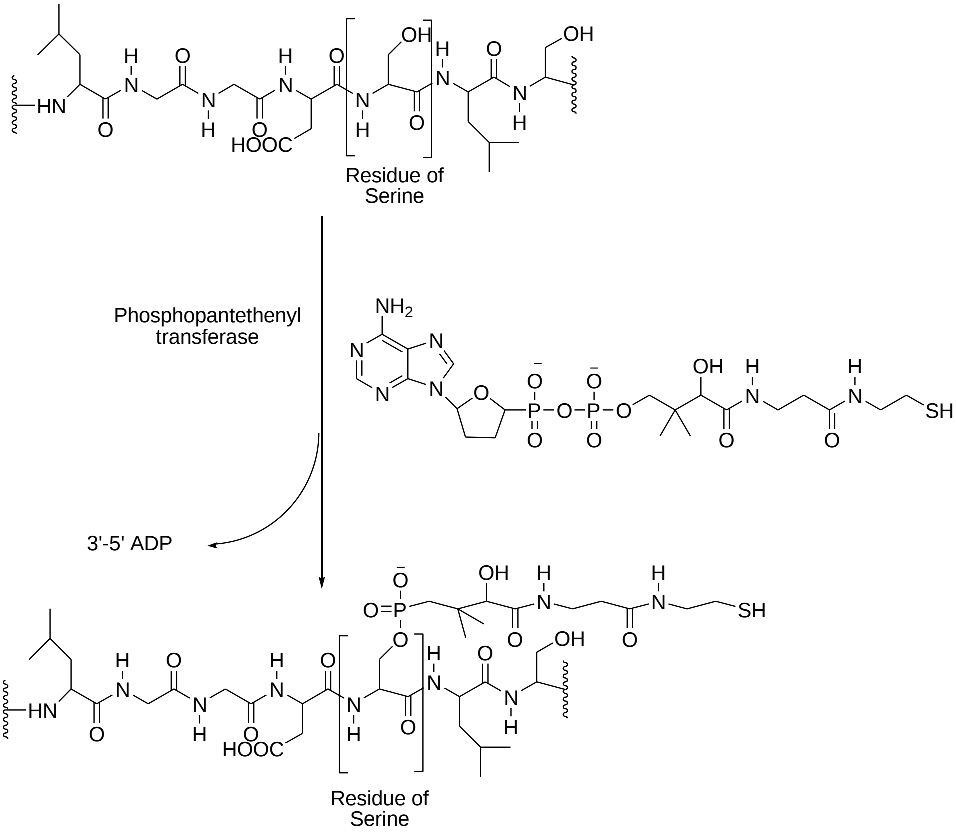
In literature, the bio-reduction of (**XL**) acid was reported using whole cell of *Pyrococcus furiosus*[179] but 3-phenylpropan-1ol (**VII**) was obtained. On the contrary the preparation of this aldehyde **VI** using CAR obtained from *Micobacterium marinum* was not previously described, even if some data with different CAR was found in the literature[180]

3.11.2 Production of CAR enzyme and catalytic cycle

The selected CAR enzyme was expressed into a host cell of *Escherichia coli*. A phosphopantetheinyl transferase (PPTase) from Bacillus subtilis was co-expressed alongside CAR because it was earlier found that PPTase is essential for maximum activity[176].

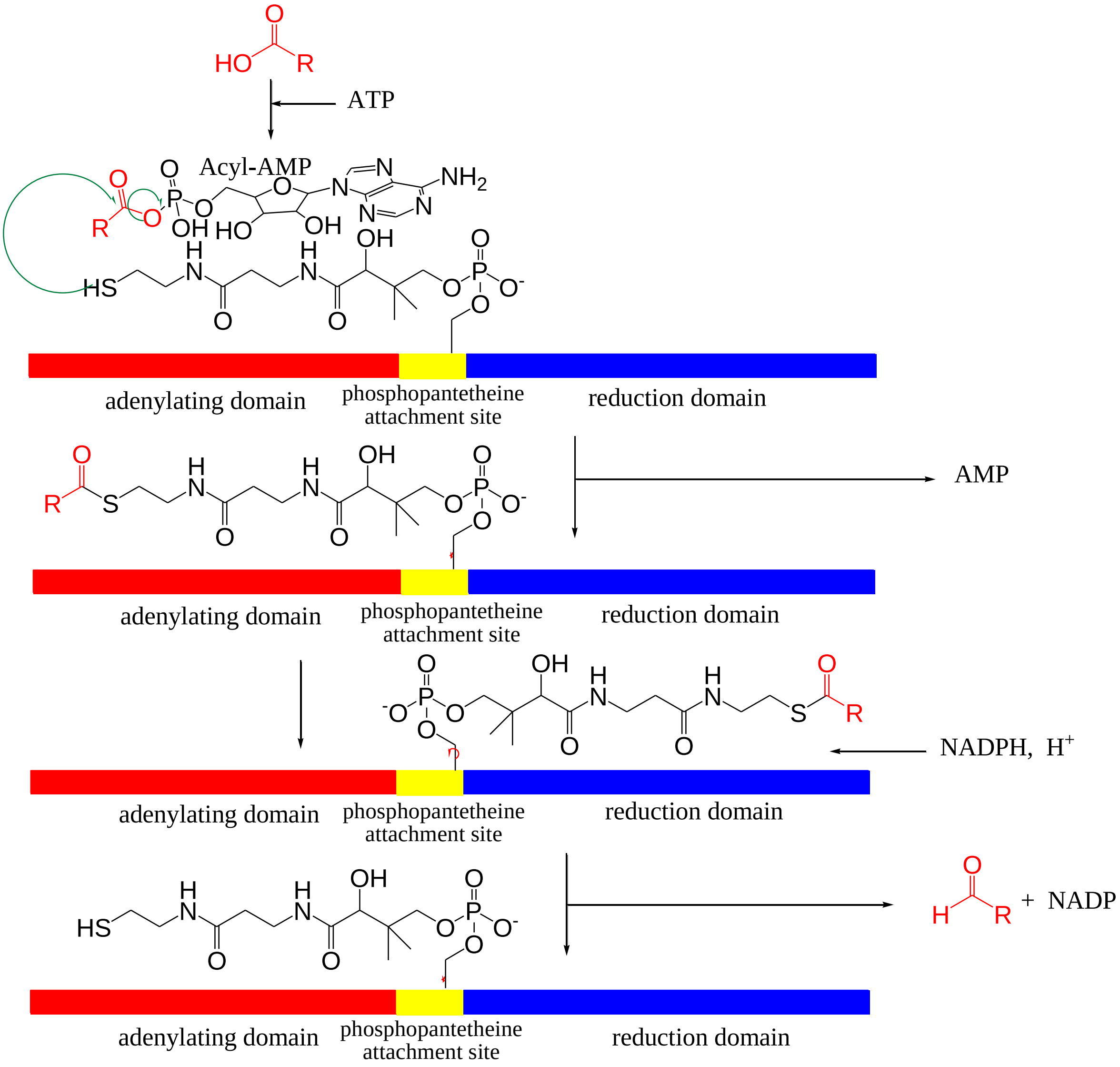
Phosphopantetheinyltransferase catalyzes the post-translational modifications of proteins by covalently attaching the 4-phosphopantetheine moiety of CoA usually to a residue serine of an apoprotein. The role of phosphopantetheinyltransferase has been well documented in fatty acid and polyketide biosynthesis and in non-ribosomal peptide preparation[176]. PPTases SFP have been classified according to their specificity for a given protein:

1. For acyl carrier proteins involved in fatty acid and polyketide biosynthesis.
2. For aryl-carrier proteins and peptide carrier proteins involved in non-ribosomal peptide and siderophore biosynthesis.
3. A PPTase SFP that exist as an integrated domain of fatty-acid synthases
4. A subclass of PPTase that is related to the Bacillus subtilis PPTase SFP and shows higher catalytic efficiencies with carrier protein involved in primary metabolism[176].



Scheme 3.11.3. Transfer of a phosphopantetheine moiety from CoA to an acceptor apo-CAR. The residue of Serine is maintained.

In the reference[176] a possible catalytic cycle for the reduction of carboxylic acids into aldehydes is described. In the first step, the carboxylic acid is adenylated into the adenylating domain in the presence of ATP affording acyl-AMP. In the second step, a nucleophilic attack of the phosphopantetheine thiol at the carbonyl group of acyl-AMP releases AMP and forms a covalently bounded acyl thioester. In the third step, the acyl thioesterphosphopantetheinyl "arm" swings from the adenylating domain to the reduction domain. Here, the thioester is reduced by NADPH, so releasing the aldehyde, NADP$^+$, and free enzyme ready for another cycle.



Scheme 3.11.4. Ffunctioning of CAR enzyme

3.11.3 Obtained results and discussion

As already reported, CAR enzyme was obtained by fermentation of a modified *Escherichia coli* strain. Into this microorganism, two plasmids were inserted, one containing the information to express CAR enzyme and the other being able to express phosphopantetheinyl transferase (from here denominated SFP). Furthermore the plasmids had the resistance for ampicillin and chloramphenicol. These two antibiotics would have been introduced during the fermentation so permitting only to resistant cells to grow. At the end of fermentation, the OD (optical density) of the culture was checked at 600 nm. The obtained value was 8.25. With this value according to the protocol, the amount of required buffer to extract the enzyme from cells is determined by the following formula:

$$\frac{(32.2 \times OD\ of\ the\ culture)x(Volume\ (mL) of\ cells\ in\ aliquot)}{(1000)}$$

$$= Volume\ buffer\ needed\ in\ mL$$

The lysis buffer is composed by a 50 mM HEPES solution (pH=7.5), 2 % (v/v) n-Hexane and the final concentration of PMSF (phenylmethanesulfonylfluoride) is 1 mM. PMSF is added to block the activity of some proteases that could destroy CAR outside of the cell. N-Hexane is added to facilitate removal the membrane phospholipids and favor the extraction of the enzyme. After calculation, the needed total volume buffer was about 2.7 mL.

Cells lyses were made by using ultra-sounds equipment and the obtained cell free extract was analyzed by SDS-PAGE (Sodium dodecyl sulfate–polyacrylamide gel electrophoresis):

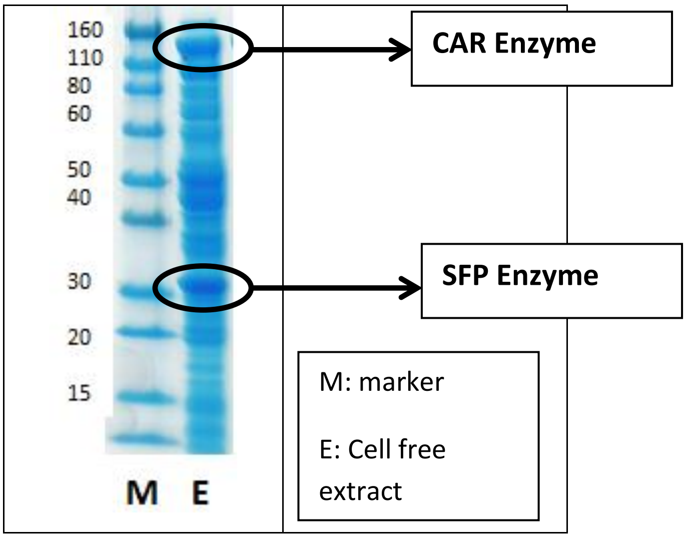


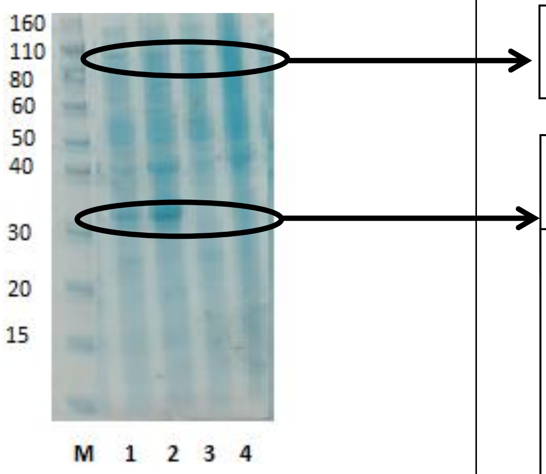
Figure 3.11.1. Cell free extract (CFE) analysis by SDS-PAGE

The presence of the two enzymes was possible by comparison with the molecular weights previously reported[37].

To demonstrate that the observed activity of aldehyde production was dependent by enzyme CAR and not by other enzymes the following two control experiments were performed.

In the first one, fermentation of *Escherichia coli* cells containing the plasmid vector encoding the production of CAR, devoid of the gene sequence capable of producing such enzyme was carried out. In the second test, fermentation of *Escherichia coli* cells containing both empty plasmids vectors, normally enconding CAR and SFP enzymes, was performed.

The obtained cultures were treated in the same way previously described and in the Figure 3.11.2 the analysis is reported:



Absence of CAR Enzyme

SFP Enzyme is present only in 1 and 2 samples

M: Marker

1: Only SFP vector plasmid (CFE)

2 Only SFP vector plasmid (centrifuged pellets)

3: Test with both empty plasmid (CFE)

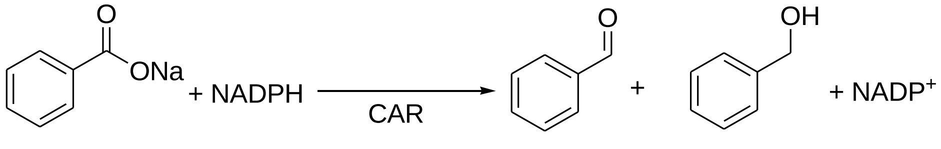
4: Test with both empty plasmid (centrifuged pellet)

Figure 3.11.2. Analysis of CFE and cell pellets obtai on of *Escherichia coli* culture

After centrifugation of sample, the compositions of CFE and cells presents as bottom pellets were checked.

From the first control experiment, only SFP spot was detected in Cell free extract and centrifuged pellets (line 1 and 2 of Figure 3.11.2) while from the second control experiment, no spot corresponding to CAR o SFP enzymes in CFE or centrifuged pellets was found (line 3 and 4 of Figure 3.11.2).

The control of the enzyme activity was initially performed using sodium benzoate (**LXXVI**) instead of benzoic acid which had been described as suitable for this reaction by adopting a precedent well tested internal protocol[175]:

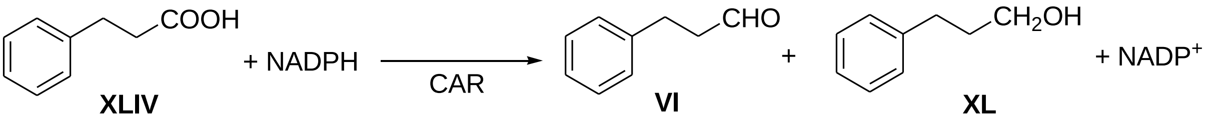


LXXVI **LXXII** **LXXVIII**

Scheme 3.11.5. Reduction of sodium benzoate (LXXVI) catalyzed by CAR enzyme

The reaction was performed as described at paragraph 5.15.9 of experimental part.

As expected, after 5 h, 67 % conversion of sodium benzoate (**LXXVI**) was detected and 45 % of benzaldehyde (**LXXII**) and 22 % of benzyl alcohol (**LXXVIII**) was found. This result means that the CAR enzyme was active in the reduction of this carboxylic group but the unexpected formation of (**LXXVIII**) could be due to the presence of alcohol dehydrogenase as impurity enzyme. The reaction was then studied on our target substrate: 3-phenylpropionic acid (**XLIV**) and the obtained results are reported in Table 3.11.3.



XLIV **VI** **XL**

Scheme 3.11.6. Reduction of 3-phenylpropionic acid (XLIV) catalyzed by CAR enzyme

Table 3.11.3. Reduction of XLIV catalyzed by CAR enzyme

Run	Time	Conv. XLIV (%)	VI (%)	XL (%)
1[a]	5	65	62	3
	21	65	62	3
2[b]	5	38	38	---
	21	44	27	17
3[c]	5	32	32	---
	21	40	40	---

Reaction conditions: [a] **XLIV**= 0.010 g (0.06 mmol); NADPH = 0.054 g (0.06 mmol); ATP = 0.033 g (0.06 mmol); $MgCl_2$•7 H_2O = 0.020 g; CFE = 1 mL; HEPES buffer (50 mM at pH = 7,2) = 9 mL; T = 25°C; [b] same conditions of run 1 but the solvent was 8 mL of HEPES buffer (50 mM at pH = 7.2) and 1 mL of MTBE; [c] same conditions of run 1 but 0.027 g (0.03 mol) of NADPH was used (**XLIV**/NADPH molar ratio 2/1), and 0.0036 g (0.06 mmol, d=0.785 g/mL) of propan-2-ol was used.

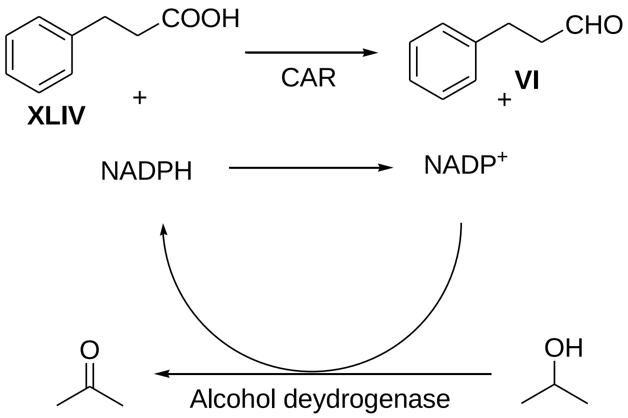
In the run 1, the formation of **XL** was also detected and this result could confirm the presence of alcohol dehydrogenase into the CFE. It was also observed that after 5 h the conversion did not increase probably due to the deactivation of CAR enzyme. As matter of fact, in another test, not reported in Table 3.11.3, other NADPH was added after 21 h, but the conversion did not increase.

To try to improve the reaction and to increase the selectivity it was decided to perform an experiment under biphasic condition, adding 10 % (v/v) of MTBE to the aqueous reaction mixture (run 2, Table 3.11.3). As matter of fact, very often, enzymatic reactions are improved if they are carried out in a biphase water/organic solvent[181]. The reason for this is that the organic solvent solubilizing reagents and/or products may reduce the impact of potential enzyme poisoners that are removed from the direct contact with the enzyme while the reaction occurs at the interface.

It was observed that the conversion after 5-21 h was less than experiment performed in pure HEPES buffer (run 2 compared to run 1, Table 3.11.3) but it was also observed higher formation (17 %) of **XL**.

The result showed an expected slowdown of bio-catalyzed reactions rate but without preserving the activity of the enzyme CAR. The greater amount of alcohol **XL** could probably be due to the fact that the activity of alcohol dehydrogenase enzyme was not adversely affected by working under biphasic conditions.

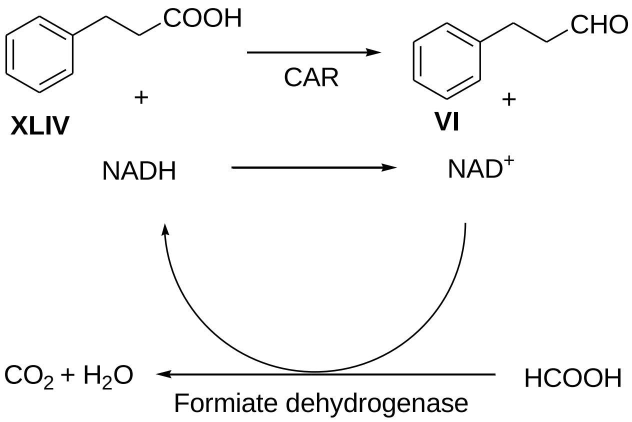
In previous two runs, a stoichiometric amount of NADPH co-factor was used. In order to improve the sustainability of reaction, it would be therefore necessary to regenerate the reduced form of the co-factor, i.e. NADPH from $NADP^+$. It is in principle possible using another bio-catalyzed reaction where a cheap substrate may be oxidized using NADP$^+$ as co-factor. Since an alcohol dehydrogenase appeared to be present in the reaction mixture it was decided to use propan-2-ol as sacrificial reagent (Scheme 3.11.7):



Scheme 3.11.7. Reduction of XLIV catalyzed by CAR enzyme in presence of sacrificial reagent (propan-2-ol)

A promising result (run 3, Table 3.11.3) was observed. In fact it was possible to work with a sub-stoichiometric amount of the cofactor and the reaction was selective toward the aldehyde **VI**, with no formation of **XL**. Furthermore, the activity of CAR enzyme seemed to survive after 5 h even if the conversion after 21 h was still lower than run 1, Table 3.11.3. In future, further experiments have to be made to try to improve this result for a possible scaling-up. One possibility would be surely that of verifying other biocatalyzed reaction coupled with CAR to regenerate the co-factor.

As matter of fact a further reduction experiment using a sub-stoichiometric amount of NADH as cofactor was tried in the presence of a formate dehydrogenase added to reaction mixture together with formic acid as sacrificial reagents (Scheme 3.11.8):



Scheme 3.11.8. Reduction of XLIV catalyzed by CAR enzyme in presence of formic acid as sacrificial reagents

Although it was known that CAR enzyme prefers NADPH as cofactor instead of less expansive NADH, in literature[37] it was reported that the use the latter cofactor would still be possible even if with a slower reaction rate. It was confirmed by experiment, not shown here, where NADH was used in stoichiometric amount, following the activity of CAR enzyme by a spectrophotometer.

Time (s)	**Abs**	**Abs**
0	0.907	1.172
30	0.566	1.044
60	0.3	0.887
90	0.106	0.795
120	0	0.694
150	-0.05	0.562
180	-0.069	0.485
210	-0.069	0.394
240	-0.072	0.307
270	-0.079	0.244
300	-0.076	0.185
330	-0.073	0.128
360	-0.073	0.083
390	-0.073	0,043
420	-0.073	0.02
450	-0.078	0
480	-0.08	-0.015
510	-0.073	-0.024
540	-0.072	-0.029
570	-0.072	-0.034
600		-0.037
630		-0.038
660		-0.041
690		-0.043
720		-0.044
750		-0.045
780		-0.045

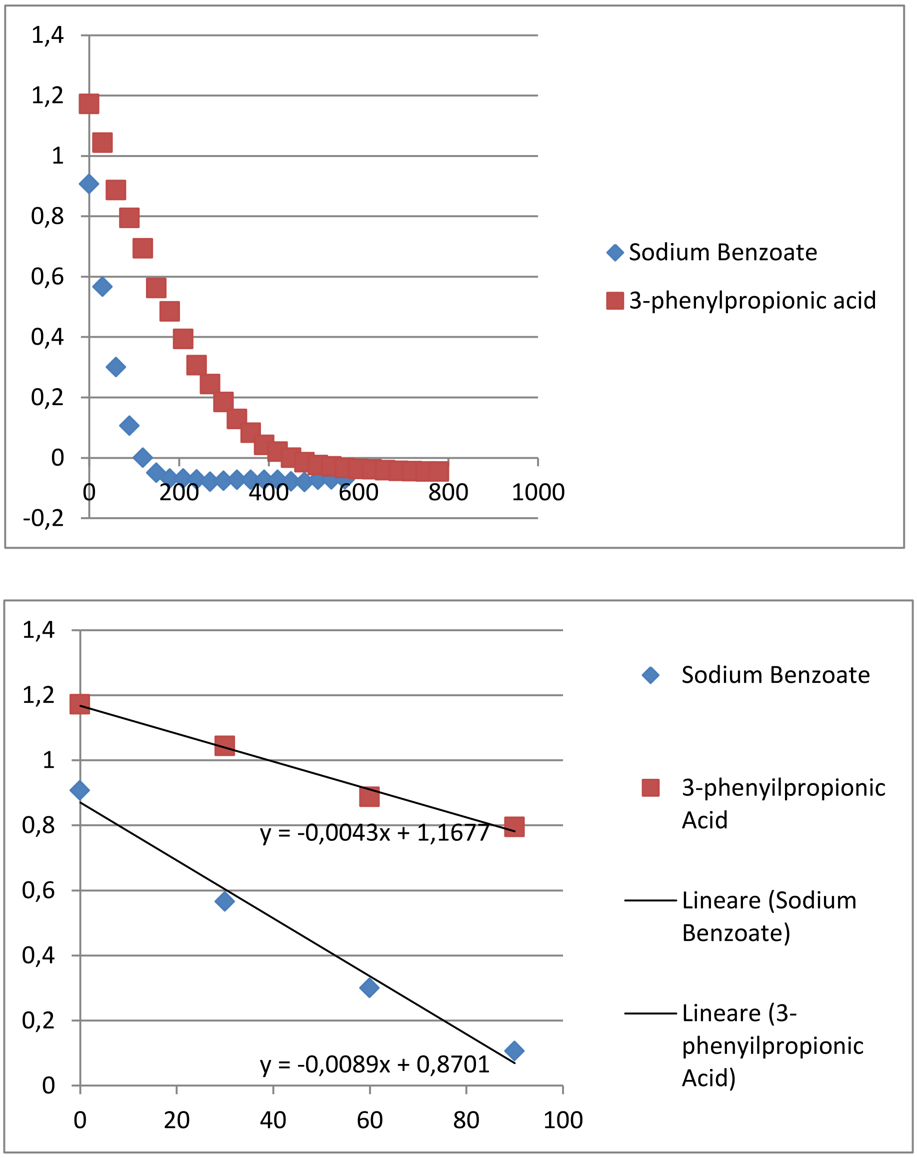


Figure 3.11.3. Activity check of CAR enzyme using LXXVI and XLIV as substrate and NADPH as cofactor

Time (s)	Abs	Abs
0	1.742	1.703
30	1.726	1.667
60	1.700	1.651
90	1.682	1.616
120	1.661	1.593
150	1.631	1.564
180	1.609	1.528
210	1.584	1.497
240	1.557	1.462
270	1.529	1.433
300	1.503	1.398
330	1.468	1.365
360	1.449	1.344
390	1.422	1.310
420	1.395	1.281
450	1.370	1.256
480	1.342	1.236
510	1.312	1.211
540	1.284	1.185
570	1.257	1.155
600	1.232	1.134
630	1.204	1.104
660	1.166	1.073
690	1.142	1.044
720	1.113	1.018
750	1.098	0.990
780	1.074	0.962
810	1.069	0.934
840	1.021	0.907
870	1.000	0.879
900	0.967	0.858
930	0.950	0.820
960	0.924	0.796
990	0.898	0.771
1020	0.877	0.747
1050	0.854	0.718
1080	0.830	0.691
1110	0.807	0.667
1140	0.781	0.640
1170	0.761	0.615
1200	0.738	0.590

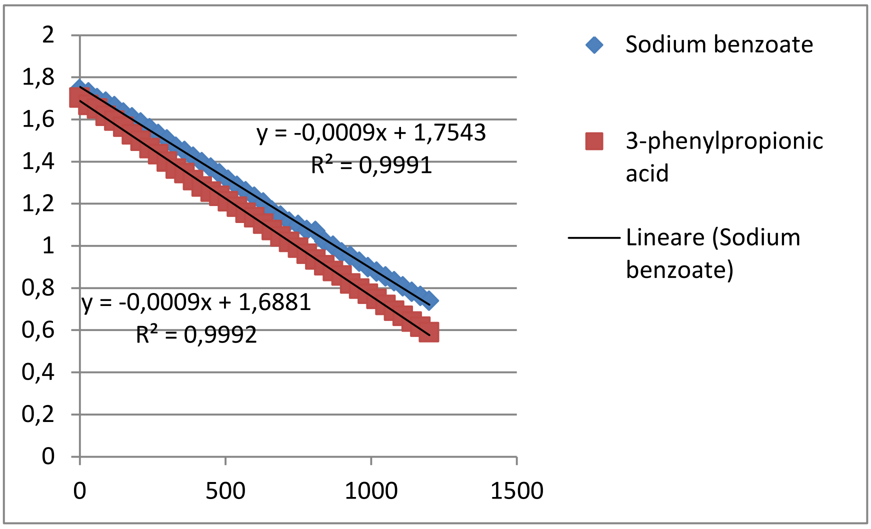
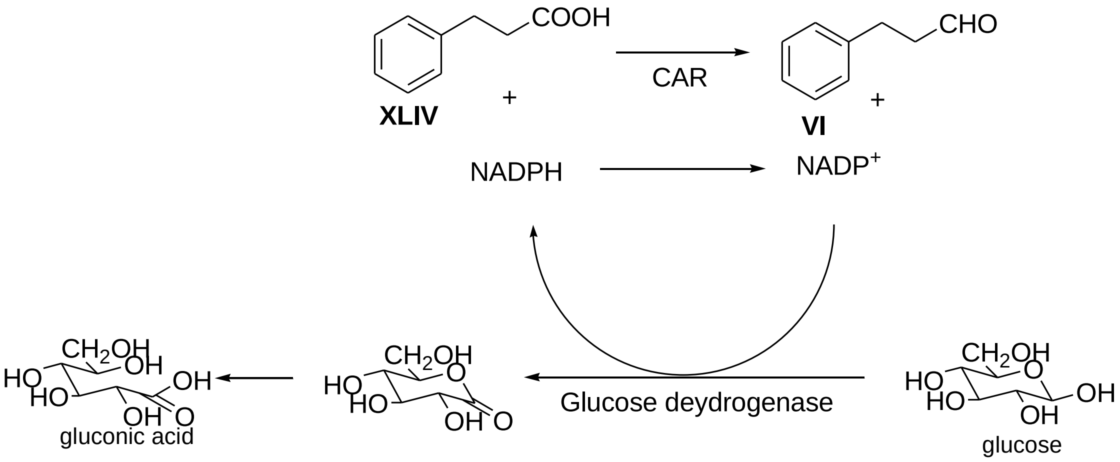


Figure 3.11.4. Activity check using XLIV and VI acid as substrate and NADH as cofactor

From the obtained data (Figures 3.11.3 and 3.11.4), it is clear that the reaction with sodium benzoate (**LXXVI**) in presence of NADPH as cofactor is faster than when **XLIV** is used, so confirming experimental results obtained when the reduction was carried out in flask, and that the reaction is slower when the NADH is used, but occurs.

However it was necessary also to make a preliminary blank spectrophotometric test to exclude any activity of formate dehydrogenase on **XLIV**. No activity was indeed detected by the test even if this enzyme resulted very efficient in transforming formic acid into CO_2 and water, either under the typical condition of this reaction (paragraph 5.15.9.4) or using the best CAR enzyme reaction condition. Unfortunately the final experiment according to the scheme 3.11.8, gave no 3-phenylpropanal (**VI**) as product and the 3-phenylpropionic acid (**XLIV**) resulted untouched.

In the future it would be necessary to verify the possibility to regenerate the NADPH, coupling CAR reaction with the biocatalyzed oxidation of glucose mediated by glucose dehydrogenase (Scheme 3.11.9). As matter of fact, it is well known that the regeneration of NADPH is performed efficiently using this strategy[181] even if this protocol has not been described in combination with a CAR reduction until now.



Scheme 3.11.9. Reduction of XLIV catalyzed by CAR enzyme in presence of Glucose dehydrogenase

3.11.4 Purification of enzyme by Ni-NTA-cromatography (Nickel-nitrilotriacetic acid-chromatography)

All above described experiments were performed using crude CAR enzyme (Cell free extract) that from sustainable point of view will be the preferred industrial solution. However, as shown before, this crude extract contains undesired alcohol dehydrogenase as impurity. Therefore it was compulsory to try to purify CAR enzyme before repeating above experiment to check if this purification could gave any advantage.

The purification of CAR had been described using a Ni-NTA chromatography[37]. This technique is suitable for recombinant proteins in which there are 6 Histidine residues. In this case both CAR and SFP enzymes have this characteristic since DNA coding the residue of histidine had been inserted near the DNA coding the desired bio-catalysts. The principle of this chromatography purification is based on the interaction of Ni-NTA with 6-histidine residue (Figure 3.11.5); the protein remains blocked on the column while impurities are removed with washings; finally the protein is removed from the column using imidazole which causes the dissociation of the bond Ni-histidine.

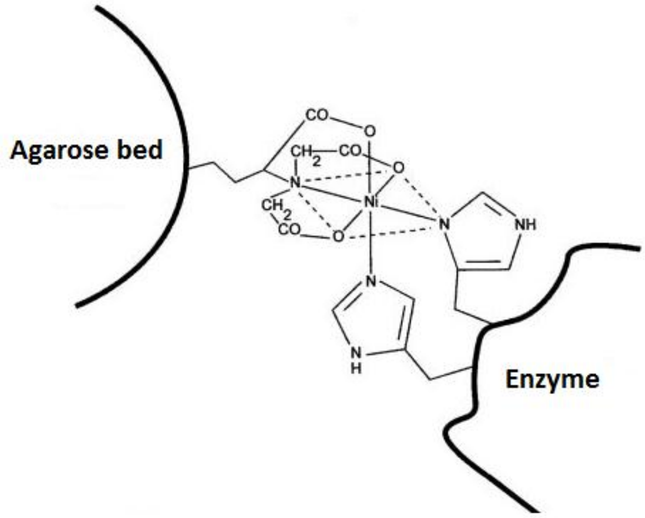
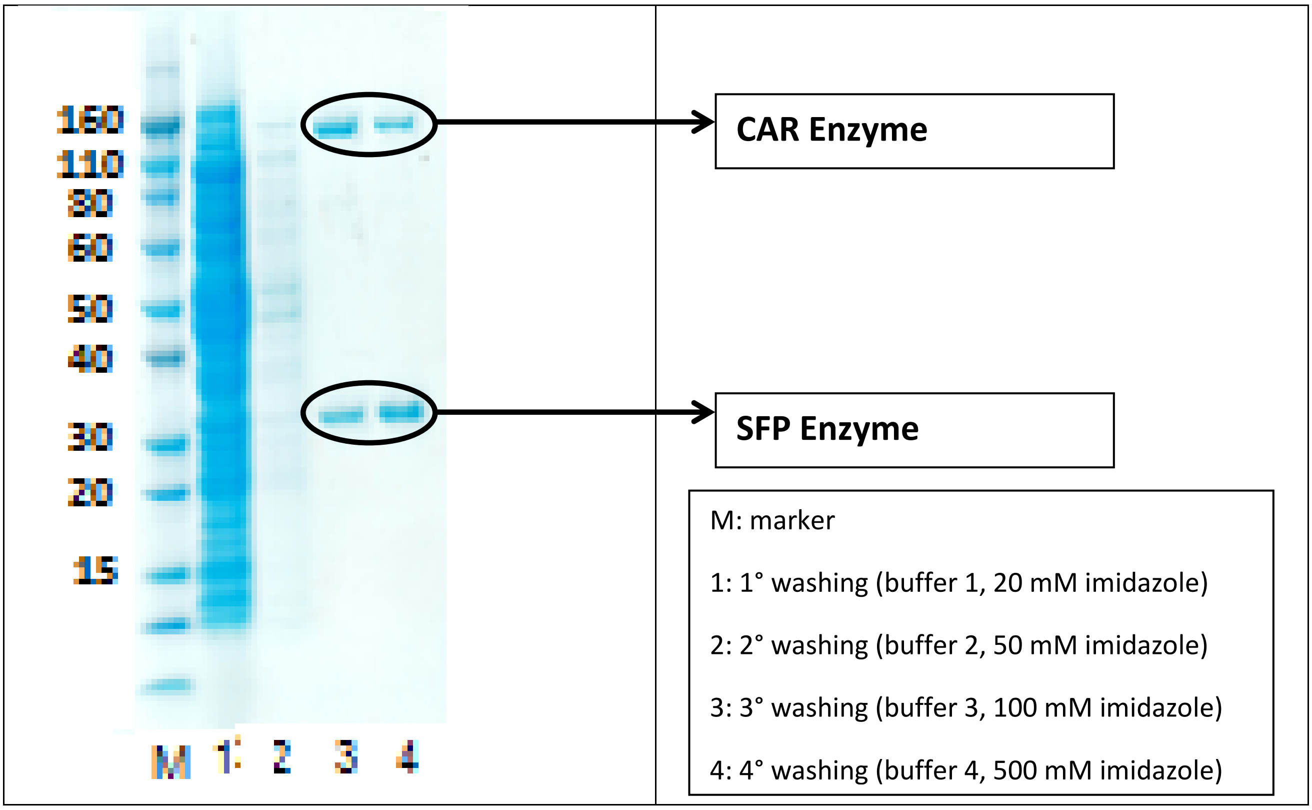


Figure 3.11.5. Interaction between the modified Ni-NTA and the histidine residues on Enzyme[182]

As matter of fact imidazole is competitive with the imidazole ring of histidine and solution from 50 up to 500 mM can dissociate the protein from the Ni-NTA agarose support.

After fermentation, Cell Free Extract was recovered by ultrasounds equipment as previously reported (paragraph 3.11.3) and the procedure for purification reported on experimental parts was followed.

The obtained filtrate was checked on SDS-page:



M: marker

1: 1° washing (buffer 1, 20 mM imidazole)

2: 2° washing (buffer 2, 50 mM imidazole)

3: 3° washing (buffer 3, 100 mM imidazole)

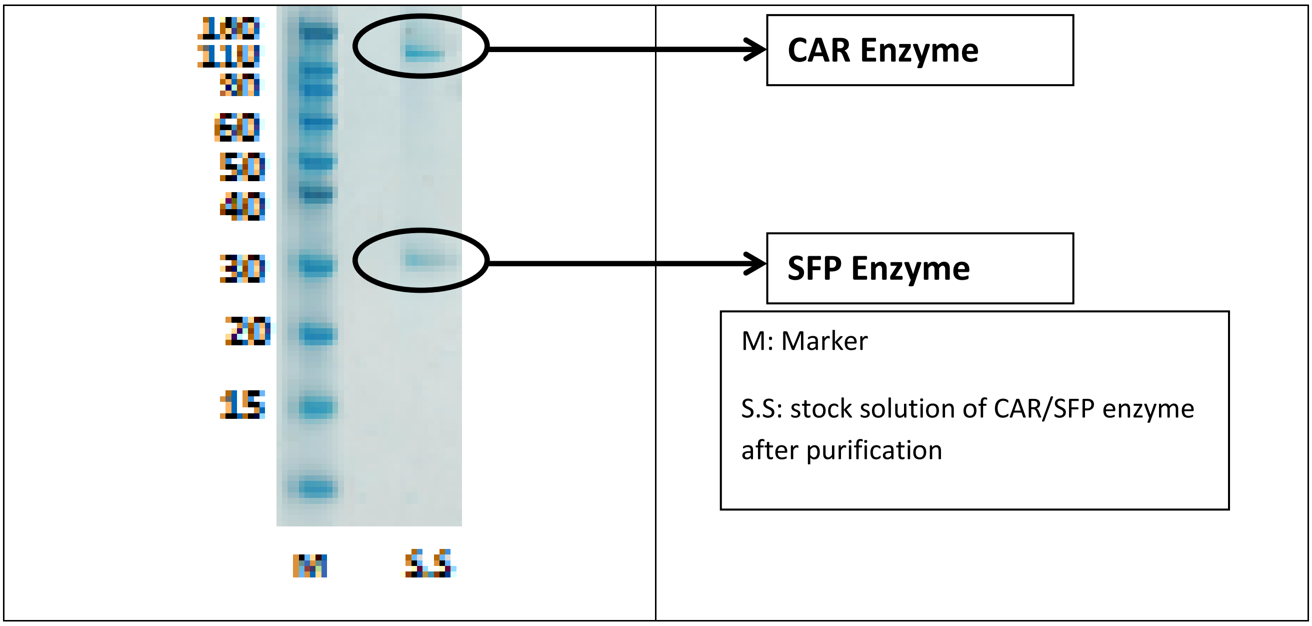
4: 4° washing (buffer 4, 500 mM imidazole)

Figure 3.11.6. SDS.PAGE analysis of eluate obtained from washing of purification column

It is possible to observe that into the first filtrate, obtained by using of buffer 1, there are some spots corresponding to different protein molecules, but the spots corresponding to CAR enzyme and SFP enzyme are not present. Using the solution at 50 mM of imidazole (buffer 2), it is not possible to remove the enzymes from column (the concentration of imidazole is not enough to break the ligand between enzymes and Ni) but using the buffer 3 and 4, CAR and SFP enzymes were recovered in pure form. Only the filtrate obtained from washing with buffer 3 was kept because the spot corresponding to CAR enzyme is more intense than the spot corresponding to SFP enzyme. Into the filtrate obtained from using of buffer 4, there are both CAR and SFP enzymes but the spot of SFP is more intense and for this reason this solution was discarded.

The solution obtained from buffer 3 was further purified by dialysis (cut off < 10000 Dalton) and subsequently treated as reported in procedure described at paragraph 5.14.4.3.

At the ends of these operations the pure solution of CAR and SFP enzymes was obtained and was 4stocked at -80°C.



M: Marker

S.S: stock solution of CAR/SFP enzyme after purification

Figure 3.11.7. SDS-PAGE analysis of stock solution: only CAR and SFP presence was detected

Preliminary test conducted by using spectrophotometer, allowed verify that after this purification procedure that CAR enzyme was still active to reduce carboxylic acid such as 3-phenylpropionic acid (**XLIV**). Furthermore the formation of alcohol **XL** was not detected. This result is a further confirmation that the formation of undesired alcohol **XL** was due to the presence of impurity in Cell Free Extract (CFE).

A goal originally intended that was not possible to reach was to determine in a precise manner the concentration of CAR and SFP enzymes in pure stock solution and in the raw Cell Free Extract (CFE). The exact determination of enzyme concentration would have required a HPLC-quantitative analysis on a special HPLC column that was not available. When this data will be obtained, it will also possible to compare the activity of CAR in CFE with pure CAR. These are planned activities in the near future.

3.12 Acid Catalysis

As already reported in Chapter 1, acid catalysis plays a key role in chemical industry. However the use of traditional Brønsted and Lewis acid is often characterized by the high amount wastes formation and in certain cases by low reaction selectivity. In order to improve these aspects, many solutions have been developed and, for example, the use of functionalized polymers was deeply investigated. Among these polymers, one thoroughly investigated and used as Brønsted and Lewis acid catalyst is Nafion®, which was developed by W. Grot at DuPont in 1960s. It is synthesized by the free radical copolymerization free radical between tetrafluoroethylene and perfluorinated vinyl ether monomers:

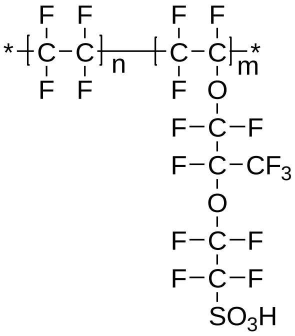


Figure 3.12.1. Structure of Nafion®

Nafion® was the first polymer belonging to the ionomers class. Its first use, as separation membrane, was in electrolytic cells to produce chlorine and caustic soda. Subsequently, its use was expanded to produce membranes for PEM fuel cells. In fact before mid-1960’s, synthetic polymers attracted attention largely as materials rather than as organic molecules in their own right. The use of Nafion® , as Brønsted acid, and its salts (such as Cr, Ce, Hg) are widely mentioned in literature as catalyst for alkylation, acylation, nitration, sulfonation, polymerization, oxidation, hydration and other reactions[183,184]. Major drawbacks of this material is the very low surface area and the fact that the activity of this material in non-swelling solvents may be very low. This in turn has limited the application in Brønsted acid catalysed reactions. In order to increase the acid site accessibility of Nafion® resin-based catalysts, a class of solid acid catalyst-based upon a high surface area Nafion® resin silica nanocomposite, has been then developed, where nanometer sized Nafion® resin particles are entrapped within a highly porous silica network[185,186]. The property of some Nafion® salts as Lewis acid have been less investigated; it is then to underline that when Nafion® salts are used, they were prepared by cation exchange from the corresponding Nafion®-K salt, and this procedure produces aqueous salt wastes.

Very recently, the preparation of Nafion®-Fe was reported[187]. This Lewis Acid catalyst was used with success in a Strecker reaction. Also in this case Nafion®-Fe is prepared by cation exchange reaction starting from Nafion®-K and using $FeBr_3$ in dichloroethane at reflux. After the preparation, the catalyst is washed by dichloroethane, by water to remove the excess of $FeBr_3$ and acetone and finally it is dried at room temperature under vacuum for 16 hours.

During this work of thesis the properties of another polyfluorinated polymer and its salts, as Brønsted acid as well as Lewis acid catalyst, were investigated. This polymer is Aquivion®PFSA (PerFluoroSulfonic Acid, and from here referred as Aquivion®) and is produced by Solvay Specialty Polymers S.p.A. It is synthesized by reaction between Tetrafluoroethylene and suitable Sulfonyl vinil ether by emulsion free radicals copolymerization:

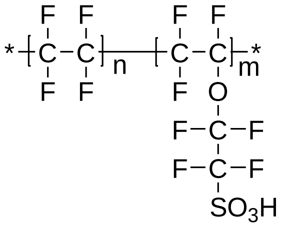


Figure 3.12.2. Structure of Aquivion®

The major difference between Nafion® and Aquivion® is the length of the side chain. Nafion® is classified as long side chain ionomer and Aquivion® as short side chain ionomer. The length of the side chain influences the crystallinity of the polymer (the shorter side-chain allows, at a given -SO_3H loading, a higher polymer crystallinity and thus a higher mechanical resistance) and its thermal behavior (the softening temperature of the short side chain ionomer is lower than that of its long side chain congeners thus permitting to work at higher temperature, if necessary[188,189]. Furthermore, the very poor porosity of Aquivion® powder could reduce or prevent reactions on some substrates but also permit a better selectivity. However, it is also important to note that the length of side chain might affect the availability of the acid site. During this work of thesis, the possibility to prepare of some metallic salts starting from Aquivion® was considered. This salification might cause a rearrangement of the polymer structure so to modify the substrate access towards the catalytic site.

Furthermore it was also assumed as hypothesis that the formation of some metallic salts could be easier with Aquivion®and cause a rearrangement of the polymer structure so to overcome or reduce the problem, if any, of limited access to the catalytic site.

3.12.1 The use of Lewis Acid catalyst in organic synthesis

As already mentioned in the introductory paragraph 1.5. on acid catalysis, Lewis Acid (LA) catalyzed reactions are of great interest in organic synthesis[190]. A wide variety of these reactions have been developed and applied to the synthesis of natural and unnatural compounds. As previously reported, in 1991, the use of water-compatible lanthanide triflates, as Lewis Acid, was described[23]. After this report, many triflates have been developed and applied. An interesting advantage is the fact that these catalysts can be used in catalytic amount, unlike many traditional Lewis acids, in some reactions such as Friedel-Crafts acylation and they can also work in organic solvents/water mixtures. Another advantage is the possibility sometimes to recover them from the crude reaction mixture and to reuse. However, rare earth metals are expensive and for this reason other triflates have been investigated such as Zn, Fe, Ga and In triflates[23], being these metals more available in nature and much cheaper than rare earth metals.

In this work of thesis, the preparation of Aquivion®salt species, Aquivion®-Me and in particular Aquivion®-Fe, -Ga or -In, never investigated before, was studied using a simple and more sustainable approach, according to the following Scheme 3.12.1:

$$3\ \text{-RSO}_3\text{H} + \text{M} \longrightarrow \text{-[RSO}_3]_3\text{M} + 3/2\ \text{H}_2$$

Scheme 3.12.1. Reaction between metal and sulfonic group of Aquivion®

It was assumed that one mole of metal can react with three moles of sulfonic groups. Actually Aquivion®, where the load of sulfonic groups is equal to 1 eq. per 870 g of polymer, was used.

The procedure to obtain Aquivion®-Me is described in detail in the experimental part (paragraph 5.14.5). The preparation was carried out at reflux temperature of acetonitrile for many hours. When Aquivion®-In was prepared, it was observed that the reaction between metal and polymer was very slow. After 48 h, the presence of unreacted Indium was detected and the polymer remained partially white. After 15 days, the polymer became grey but some Indium particles were still observed. Best results were obtained when Aquivion®-Fe and Aquivion®-Ga were prepared.

After 48 h, it was found that the polymer was yellow when iron was used and grey in the case of Aquivion®-Ga. During the preparation of these two species, residual pieces of metals were not evidenced. After analysis, adopting the method reported in the experimental part (paragraph 5.13.12), the following results were obtained:

Table 3.12.1 Determination of metal loading in Aquivion®-Fe and Aquivion®-Ga

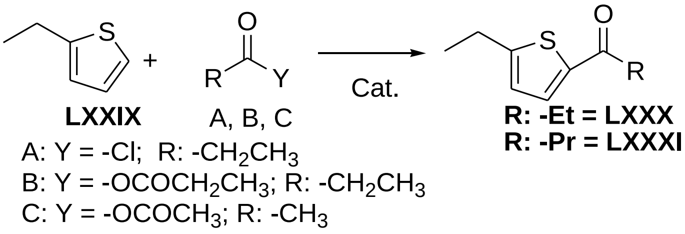
Sample	Theoretical metal loading (w/w %)	Determined metal loading (w/w %)	Theoretical metal loading (mol/mol %)	Determined metal loading (mol/mol %)	-SO_3H exchanged (%)
Aquivion®-Fe	2.140	2.053	33.33	32.01	96.03
Aquivion®-Ga	2.670	3.033	33.33	37.85	113.57

At the light of the obtained analytical results (kindly made by Specialty Polymer S.p.A staff), it is possible to say that the preparation protocol developed seems to be effective in quantitatively exchange the sulfonic acid functions available in Aquivion®, the slight differences from the 100% theoretical amount are probably due to light weighing errors of both reagents during the preparation of the catalyst. The present procedure permits to obtain in a simple way these Aquivion®salts, the reaction solvent may be easily recovered and the metallized polymer, after a rapid drying under vacuum, is ready for use as Lewis catalyst. Probably this protocol could be applied also for Nafion®-Fe and is surely more sustainable than the previously described procedure[187].

3.12.2 Applications of Aquivion®-Me and Aquivion®

In this work of thesis, the catalytic applications of Aquivion®-Me species in some Friedel-Crafts acylation and alkylation reactions and in a reductive amination are exemplified.

3.12.2.1 Acylation of 2-ethylthiophene (LXXIX)



LXXIX A, B, C

A: Y = -Cl; R: $-CH_2CH_3$

B: Y = $-OCOCH_2CH_3$; R: $-CH_2CH_3$

C: Y = $-OCOCH_3$; R: $-CH_3$

R: -Et = LXXX

R: -Pr = LXXXI

Scheme 3.12.2. Acylation of LXXIX by using of different acylation agents

Two acylating agents and different kind of catalysts were compared in this reaction. This application is interesting because it permits to obtain, in particular, 1-(5-ethylthiophen-2-yl)propan-1-one (**LXXXI**) which was originally prepared by using EtCd and 5-ethyl-2-thiophenecarboxylic acid chloride[191]. This compound is a potential fragrance, currently under investigation for some perfume applications, with a pleasant anise olfactory note and a low odor threshold.

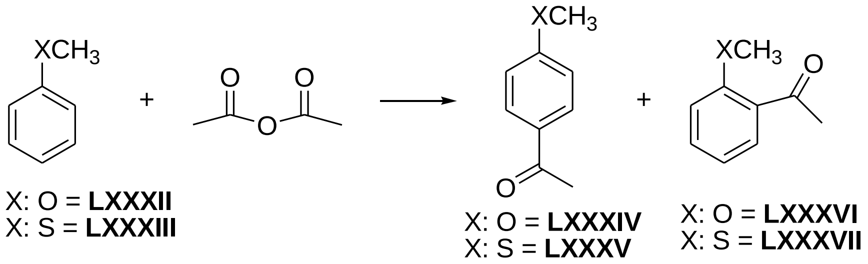
Initially a general procedure described in literature for some thiophene acylation was followed[192] but carrying out the reaction at 25 °C, in dichlomethane instead of originally used CS_2 as solvent. The catalyst, used in stoichiometric amount, was $SnCl_4$ and the acylating agent was propionyl chloride. After 2 h, a quantitative conversion of starting material into the desired product was observed and after work-up 80 % yield into **LXXXI** was obtained. This result is satisfactory but it is necessary to observe that the synthesis was carried out in a not green solvent and after reaction it was necessary to hydrolyze $SnCl_4$ so producing wastes. For these reasons, afterwards, a heterogeneous acylating catalyst, such as Envirocat EPZG^{TM}, was tested[193]. It is a commercial catalyst produced by Contract Chemicals and it is described as a zeolite doped with zinc salts, which have both Brønsted and Lewis acid characteristics[194]. The use of this catalyst permitted to work in absence of solvent and the reaction was carried out at 120°C for 4 hours using propionic anhydride as acylating agent. The obtained conversion of starting material **LXXIX** was quantitative but it was observed by GC-chromatography the formation of 7 % of an isomeric by-product, probably 3-acyl derivative. In this procedure the work up was very simple. After reaction, the catalyst was filtered off and the solution was distilled under vacuum. The obtained isolated yield was 75 %. However, a drawback of this protocol is the following: the catalyst must be activated before use and before any recycle to remove the traces of water that may affect its activity. Finally the use of Aquivion®-Me species was checked. The acylation was carried out with propionic anhydride or acetic anhydride at 120°C for 3 h in absence of solvent. The kind of anhydride gave more less the same yield. Initially, a molar ratio substrate **LXXIX**/-R$(SO_3)_3$Me 32/1 was used but subsequently it was possible to increase the ratio up to 320/1. It was observed the same results in the same time of reaction working with these two ratios, but using a reduced amount of catalyst the procedure is of course more sustainable and a more efficient mixing of the reaction mixture is obtained. To emphasize the concept, it must be remembered that working with 2 g of **LXXIX** and substrate **LXXIX**/-R$(SO_3)_3$Me ratio 32/1, for example, 1.57 g of Aquivion®-Fe were used. Working with ratio 320/1 only 0.157 g of Aquivion®-Fe were necessary. The acylation of **LXXIX** was carried out with Aquivion®-In (although part of metal was not visibly linked to the support, but in the first approach, the 4.4 % of Indium, as Aquivion® metallic salt, was assumed), Aquivion®-Fe and Aquivion®-Ga (2.67 % of Ga, as Aquivion® metallic salt was assumed). In all three cases, it was observed a quantitative conversion of starting material **LXXIX** after 3 h and an isolated yield, after distillation under vacuum, near to 75 % for **LXXX** and **LXXXI**. All catalysts were ready to use after their preparation, because activation or other treatments are not required. After reaction, the catalyst was recovered by filtration on a sintered glass filter and it was immediately reused. With Aquivion®-In and Aquivion®-Fe, the possibility to recycle catalyst was studied. The reactions were performed at 120°C with substrate **LXXIX**/-R$(SO_3)_3$Me molar ratio 320/1 and it was checked quantitative conversion of starting material **LXXIX** during the use of fresh catalyst and during the use of recovered catalyst for three times. It was not detected formation of by-product and after distillation under vacuum the isolated yield of desired ketone **LXXXI** was always 70-75 %. Despite of the presence of free Indium, it was considered for first exploratory test that all Indium was reacted with -SO_3H groups. After first use, the amount of Iron in Aquivion®-Fe was determined by Solvay Specialty Polymers S.p.A. lab by using of ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). The determined metal loading of Iron is 1.952 % w/w. The virgin Aquivion®-Fe had 2.053 % w/w metal loading, so the catalyst may be considered substantially stable.

The acylation of **LXXIX** was also carried out with Aquivion® in original acid form. The reaction was performed at 120°C for 3 h with substrate **LXXIX**/-R(SO_3H) molar ratio 10/1 in absence of solvent. The acylating agent was propionic anhydride. After 3 h of reaction, the conversion of starting material was quantitative but the isolated yield of desired product after distillation under vacuum was only 30 %. A higher amount of high-boiling point by-products was found. In a successive experiment, the ratio substrate **LXXIX**/-R(SO_3H) was increase up to 50/1 but the high formation of high-boiling point by-products was still observed.

Under these experimental conditions on this heterocycle substrate, these results show that the use of a Lewis acid as catalyst is really more efficient.

3.12.2.2 Acylation of anisole (LXXXII) and thioanisole (LXXXIII)

The acylating activity of Aquivion®–Me and Aquivion®was investigated also with other electron activated compounds. The obtained results in the acylation of **LXXXII** and **LXXXIII** are reported in Table 3.12.2.



Scheme 3.12.3. Acylation of LXXXII and LXXXIII

3.12.2. Acylation of LXXXII and LXXXIII catalyzed by Aquivion®, Aquivion®-Fe, Aquivion®-Ga and Aquivion®-In

Run	Cat.	$\frac{\text{Sub}}{-\text{R(SO}_3)_3\text{Me}}$ m.r	X	Solvent	Conv. LXXXII, LXXXIII (%)	LXXXIV, LXXXV (%)	LXXXVI, LXXXVII (%)	LXXXIV, LXXXV Isolated yield (%)
1[a]	Aquivion®-In	32/1*	S	---	28	28	Traces	10
2[a]	Aquivion®-Fe	32/1	S	---	10	10	Traces	n.d
3[b]	Aquivion®	10/1	S	---	10	10	Traces	n.d
4[c]	Aquivion®-Fe	32/1	O	---	52	50	2	33
5[d]	Aquivion®	100/1	O	---	27	27	---	n.d
6[e]	Aquivion®-Ga	320/1**	O	---	12	12	---	n.d
7[f]	Aquivion®-In	32/1*	O	A.A	---	---	---	---
8[g]	Aquivion®-Fe	32/1	O	Sulpholane	---	---	---	---
9[g]	Aquivion®-Ga	32/1**	O	Sulpholane	---	---	---	---
10[h]	Aquivion®-Fe	32/1	O	Sulpholane	---	---	---	---
11[h]	Aquivion®-Ga	32/1**	O	Sulpholane	---	---	---	---
12[i]	Aquivion®-Fe	32/1	O	Nitrobenzene	60	60	2	n.d
13[i]	Aquivion®-Ga	32/1**	O	Nitrobenzene	60	58	2	n.d
14[l]	Aquivion®-Fe	32/1	O	Nitrobenzene	62	60	2	n.d

Reaction conditions: [a] substrate **LXXXIII** = 2.00 g (0.016 mol); acetic anhydride = 1.51 mL (0.016 mol, d = 1.082 g/mL); Aquivion®-Me = 1.30 g; T = 125°C; t = 24 h; [b] substrate **LXXXIII** = 2 g (0.016 mol); acetic anhydride = 1.51 mL (0.016 mol); Aquivion®-H = 1.40 g; 125°C; 24 h; [c] Substrate **LXXXII** = 2 g (0.019 mol); acetic anhydride = 1.80 mL (0.019 mol); Aquivion®-Me = 1.65 g; [d] same conditions of run 4 but the amount of Aquivion®-H is 0.165 g; [e] substrate **LXXXII** = 2.00 g (0.019 mol); acetic anhydride 1.80 mL (0.019 mol); Aquivion®-Me = 0.155 g; [f] substrate **LXXXII** = 2 g (0.019 mmol); A.A = acetic anhydride = 25 mL; Aquivion®-Me = 1.55 g; T =140°C; t = 24 h; [g] substrate **LXXXII** = 0.1 g (0.93 mmol); acetic anhydride = 0.087 mL (0.93 mmol); Aquivion®-Fe = 0.08 g or Aquivion®Ga = 0.76 g; sulpholane = 3 mL; T = 80°C; t = 24 h; [h] same conditions of runs 7 and 8 but the temperature is 125°C; [i] substrate **LXXXII** = 0.25 g (2.3 mmol); of acetic anhydride = 0.22 mL (2.3 mmol), Aquivion®-Fe = 0.20 g; Aquivion®-Ga = 0.187 g; nitrobenzene = 3 mL; T = 80°C; t = 24 h; [l] same conditions of run 12 but the temperature is 125°C. *4.40 % of Indium as Aquivion® metallic salt was assumed; **2.67 % of Gallium as Aquivion® metallic salt was assumed.

The acylation of thioanisole **LXXXIII** was carried out by using acetic anhydride as acyl reagent, using different salt form of Aquivion® and in presence or absence of solvent. In all cases, the reactions were checked after 4-5 hours and it was observed that after this time the conversion did not increase despite the reactions were performed for 24 h. This behavior is probably due to deactivation of catalyst. In fact, in a further experiment not reported in the Table 3.12.2 and carried out under the same conditions of run 1, after 3 h, other 3 mL of acetic anhydride were added and the reaction performed for 16 hours. After 3h, 25 % conversion into acyl derivatives **LXXXV** and **LXXXVII** was found, and after 16 h, despite the addition, no increase of the conversion was determined. It was already reported in the literature that for example carboxylic acids, when present in large amounts, adsorbed so strongly on the Nafion® catalyst surface that the aromatic substrates had no chance to react[22].

Same results were found when the acylation of **LXXXII** was studied. However, it worth noting when the reaction was carried out at 125°C, using Aquivion®-Fe and using a substrate **LXXXII**/-R$(SO_3)_3$Fe catalyst molar ratio 32/1 (run 4, Table 3.12.2), a 52 % conversion into acyl derivatives was detected with a very high selectivity to **LXXXIV** product. The reaction was also performed reducing the amount of catalyst (runs 5 and 6, Table 3.12.2). In these two cases, the activity of pure Aquivion® and Aquivion®-Ga was studied, but no appreciable result was observed.

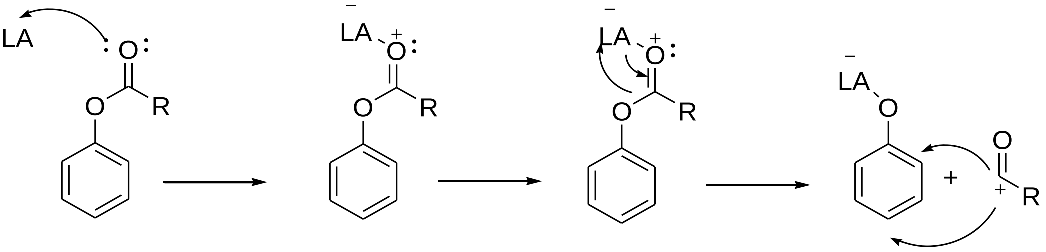
To try to increase the conversion, the reactions were checked also in presence of solvents. In fact, at the light of the obtained results, it was possible to think that the low conversion might be due to the poisoning and/or deactivation of catalyst. Furthermore, the use of a solvent could favor the swelling of the polymer and a better availability of the catalytic sites towards the reagents. For these reasons, acetic anhydride as solvent and reactant at the same time, sulpholane and nitrobenzene were used. It is worthy nothing that the only encouraging results were obtained when nitrobenzene was used (runs 12-14, Table 3.12.2). Furthermore, it is important to remark that nitrobenzene was profitably used also when metallic triflates had been used[23]. However, the toxicity of this solvent and the results here obtained (runs 12-14, Table 3.12.2) towards the reaction in absence of solvent (run 4, Table 3.12.2) do not justify its use. Probably a further check on other diluents or the use of the aromatic substrate as reactant and solvent at the same time would be useful in order to improve the catalyst activity and the yield of the reaction. Finally it is to underline that the acylation reaction was also tested on toluene as solvent and reactant at the same time (experiment not reported here). The reaction was carried out at 110°C using by substrate/-R$(SO_3)_3$Fe molar ratio 32/1, 20 mL of toluene and 1.70 mL of acetic anhydride (0.021 mol) but the formation of acyl derivatives was not observed after 24 h. This result confirms that Aquivion®-Me may be active as catalyst in Friedel-Crafts acylation reactions only on very electron rich aromatic and heteroaromatic substrates.

3.12.2.3 Acylation of Phenol (VIII) and Fries rearrangement

O-Acylation or C-Acylation products can be obtained when phenol and phenol derivatives react with anhydride or acetyl derivatives in the presence of acid catalysts. O-acylation reaction leads to obtain ester compounds while C-acylation reaction affords ortho and para hydroxyarylketones derivatives. The hydroxyarylketones are industrially important as intermediates for pharmaceuticals and as UV absorbers in plastics and coatings.

C-acylation products can be explained not only by a typical Friedel-Crafts acylation mechanism but also by an aldolic type reaction mechanism on a metal phenolate[195,196] or by acid catalyzed Fries rearrangement of O-acylation derivatives[23].

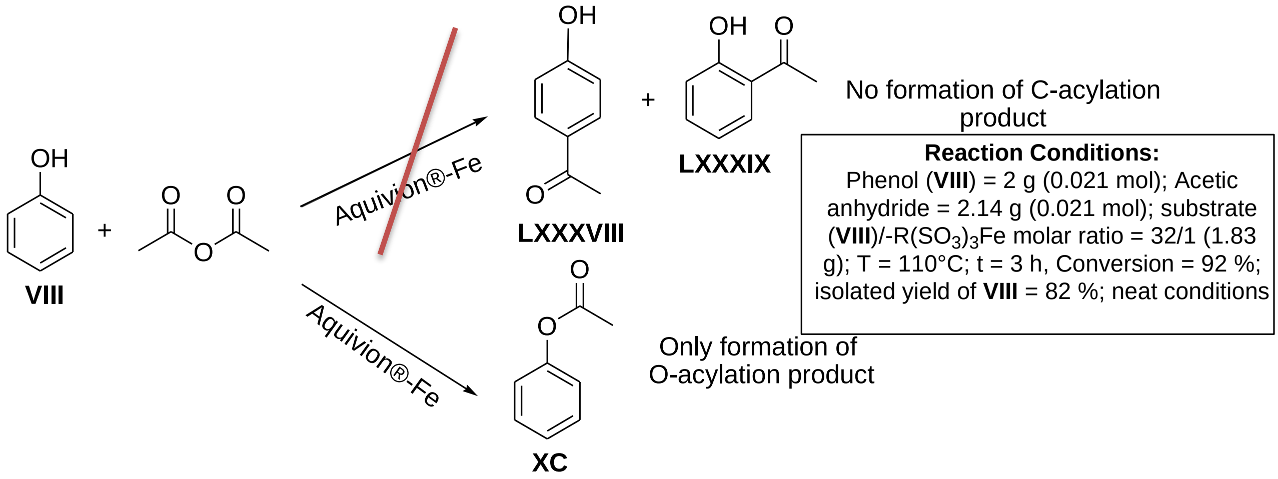
The accepted mechanism of Fries rearrangement is the following:



Scheme 3.12.4. Accepted mechanism of Fries rearrangement

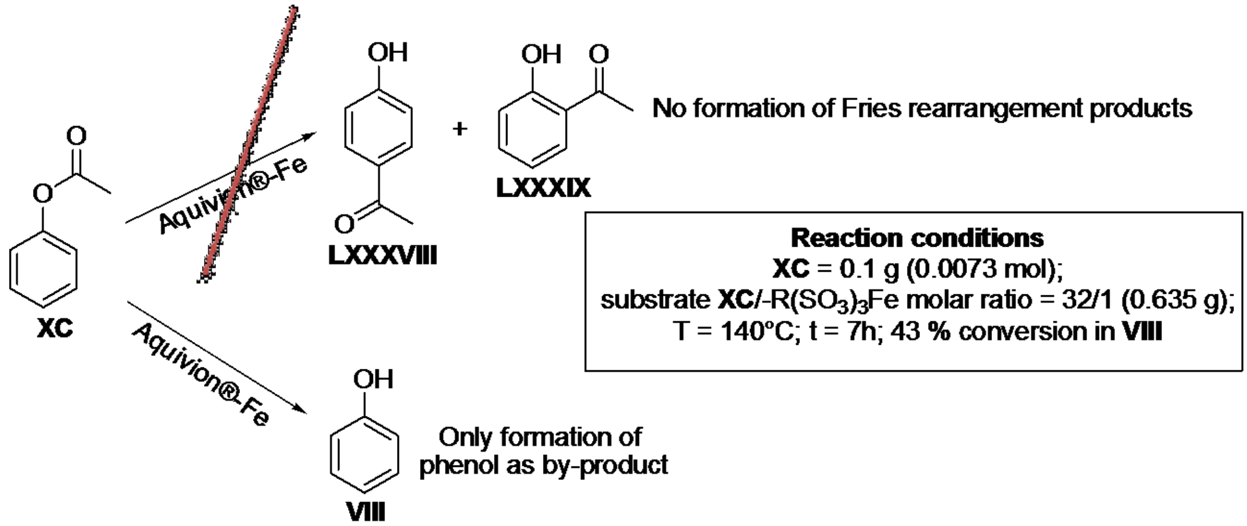
In the first step of reaction, a Lewis Acid coordinates to the carbonyl oxygen atom of the acyl group. This oxygen atom is more electron rich than the phenolic oxygen and it is the preferred Lewis base. This interaction polarizes the bond between the acyl residue and phenolic oxygen atom and the Lewis Acid rearranges the phenolic oxygen atom. This generates a free acylium carbocation which reacts in classical electrophilic aromatic substitution with the aromatic ring. The orientation of the substitution reaction is temperature dependent. A low reaction temperature favors para substitution while the ortho product prevails with high temperature. Formation of the ortho product is also favored in non-polar solvents, as the solvent polarity increase, the ratio of the para product also increases[23].

In the first approach, the direct synthesis of hydroxyarylketones was investigated by using of Aquivion®-Fe as catalyst. The reaction was carried out at 110°C in neat conditions with 2 g (0.021 mol) of phenol (**VIII**) and 1.98 mL of acetic anhydride (0.021 mol, 1.082 g/mL) for three hours, using a substrate **VIII**/-R$(SO_3)_3$Fe ratio 32/1 (1.83 g of catalyst). However, it was not observed the C-acylation but only O-acylation:



Scheme 3.12.5. Results obtained by acylation of VIII

After this time, the conversion of **VIII** was 92 % and after work-up the obtained yield of phenylacetate (**XC**) was 82 %. The direct synthesis of hydroxyarylketones **LXXXVIII** and **LXXXIX** failed and to synthetize them, the Fries rearrangement of ester was investigated. In literature the use of heterogeneous catalyst such as Nafion®/silica in Fries rearrangement is reported[23,197] and Aquivion®-Fe was tried, but only formation of (**VIII**) was observed (43 % after 7 hours):



Scheme 3.12.6. Results obtained from Fries rearrangement

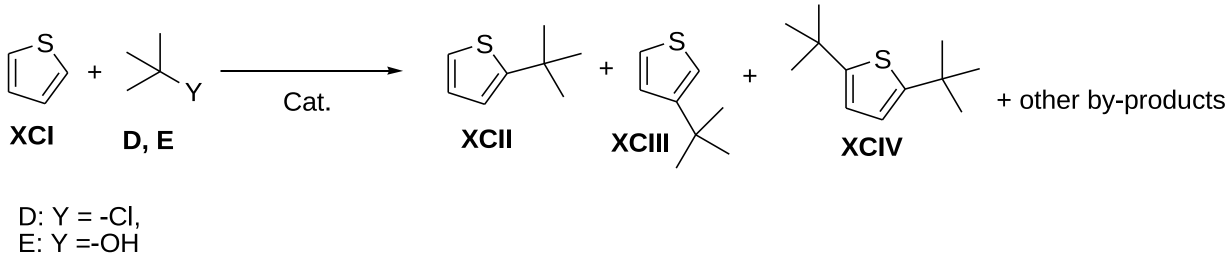
In the literature it is reported that the **VIII** could be formed by hydrolytic decomposition of starting materials or by deacylation of hydroxyarylketone **XC**[197]. The possible presence of humidity in the catalyst could favor the hydrolysis **XC**.

It is also reported that the highly reactive ketene is formed as a complementary decomposition product[198]. While the deacylation could be a reversible process, the ketene, if formed, can react irreversibly to give a large number of side-products. As already reported, the reaction is also affected by the kind of solvent; in fact, it is reported[197] that the selectivity and conversion of reaction were related to the nature of the solvent.

At the light of these considerations, other future study would be necessary to understand if an Aquivion®-Me may be active in Fries rearrangement. For example, once excluded the possibility that the moisture, eventually adsorbed by the catalyst during the storage, may be the cause of the formation of **VIII**, it would be interesting to investigate in details the nature of by-products and the effect of the steric hindrance due to the structure of polymer and to the type of metallic ion.

3.12.2.4 Alkylation of Friedel-Crafts

Besides the acylation reaction, the alkylation of Friedel-Crafts was also studied. Aquivion® and Aquivion®-Fe were used in the thiophene (**XCI**) alkylation. Initially, the reaction was carried out with tertbutylchloride or tertbutanol as reagent:



Scheme 3.12.7. Alkylation of XCI with terbutylchloride or tertbutanol

The obtained results are reported in the following Table 3.12.3.

Table 3.12.3. Alkylation of XCI

Run	Y	Cat (R-SO_3Y)	XCII (%)[c]	XCIII (%)[c]	XCIV (%)[c]	(XCII)/(XCIII)
1[a]	-Cl	Aquivion®-Fe	13	15	23	0.86
2[b]	-OH	Aquivion®	55	18	15	3.06

Reaction conditions: substrate **XCI** = 2 g (0.024 mol); tertbutylchloride = 0.22 g (0.0024 mol); Aquivion®-Fe = 0.52 g (substrate **XCI**/-R$(SO_3)_3$Fe ratio 129/1); T = 84 °C; t = 16 h; [b] same conditions of run 1 but 0.18 g (0.0024 mol) of tertbutanol and 0.52 g of Aquivion®(substrate **XCI**/-RSO_3H ratio 40/1) are used; [c] chromatographic yield determined by using toluene as external standard.

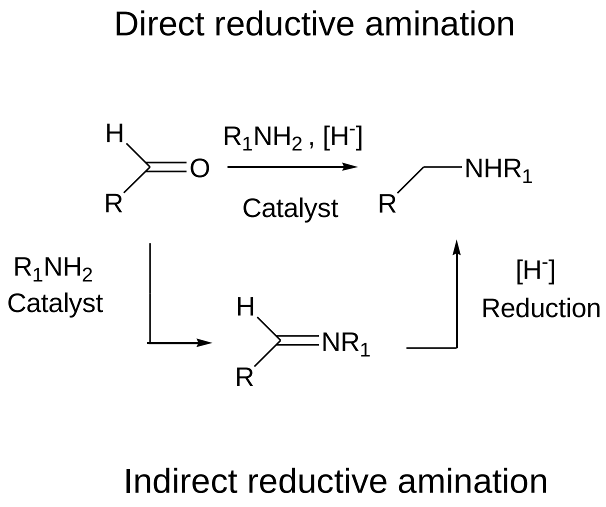
The results are expressed considering the theoretical production of single product (it was imagined that the reaction was selective for **XCII** or product **XCIII** or **XCIV** and with calibration curve the chromatographic yield was determined. The reaction carried out by tertbutanol is more selective than the reaction carried out by tertbutylchloride. Furthermore, when the reaction with tertbutylchloride was stopped, a smell of sulfur compounds and a brown mixture was detected. Conversely, the reaction with tertbutanol remained transparent as at the beginning of the reaction. This behaviour might be explained with the formation of HCl during the reaction carried out with tertbutilchloride as alkylating agent. It is well known that the **XCI** is not very stable in an acidic environment and this situation can lead to polymers and to by-products where the heteroaromatic ring is lost.

The alkylation was also carried out by isopropylchloride and isopropanol to try to obtain 2-isopropylthiophene, an important intermediate for Lioral™ (**LXI$_a$**) synthesis. (see Paragraph 3.9). Surprisingly, the formation of 2-isopropylthiophene (**L$_a$**) and other alkylated products was not detected. The reactions were carried out at 84 °C, with **XCI**/alkylating agent molar ratio 10/1 and with **XCI**/-R$(SO_3)_3$Fe ratio 129/1 or XCI/-R(SO_3)H ratio 40/1 for 16 hours.

In conclusion, Aquivion®and Aquivion®-Fe , and in particular the former catalyst, demonstrated fine activity in alkylation reaction of **XCI**. They could represent a good alternative to classical Lewis Acid, but it would be necessary to extend the study of the reactivity with other carbocyclic or heterocyclic aromatic derivatives and to try to explain the reason of non-reactivity with isopropylchloride and isopropanol. Even reaction carried out by using isopropylbromide and isopropanol in presence of $SnCl_4$ and a particular acid resin (TONSIL CO 516 E) respectively, 2-isopropylthiophene was not obtained by direct alkylation[199] further confirming the difficulty to obtain this product according to this synthetic route.

3.12.2.5 Reductive amination catalyzed by Aquivion®-Fe

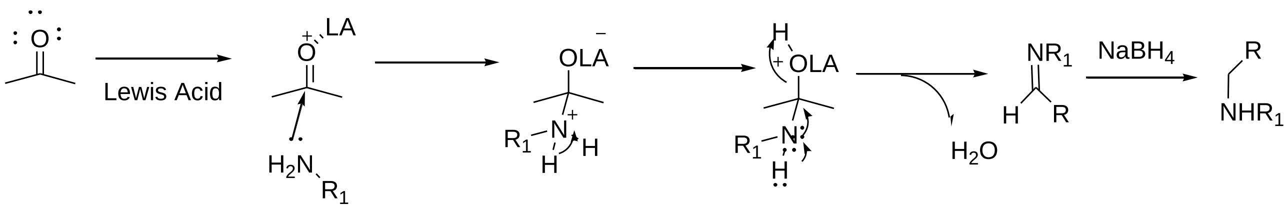
The one pot reductive amination is an important transformation in organic synthesis from process efficiency standpoint[17].



Scheme 3.12.8. Direct and indirect reductive amination

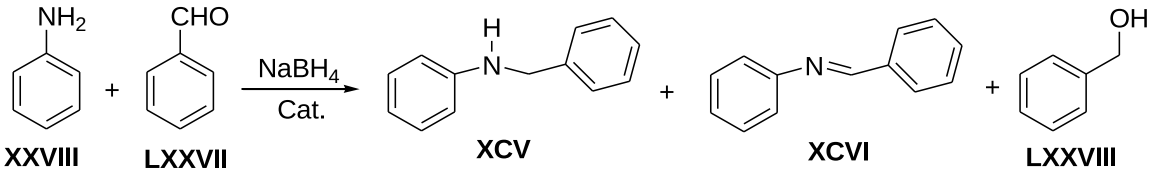
In literature, different strategies to obtain amines from aldehydes are reported, using hydrogen plus a hydrogenation catalyst or using hydrides. However to obtain the desired product with high selectivity, usually it is necessary to pre-form the imine intermediate, so reducing the side reaction of formation of the corresponding alcohol by reduction of the aldehyde. Furthermore the use of catalyzed hydrogenation could be not possible on many substrates which have other reducible functionalities. The reductive amination using sodium cyanoborohydride ($NaBH_3CN$) as the reducing agent is especially called the Borch reaction[200] and has high generality. $NaBH_3CN$ is a relatively weak reductant which works under mildly acidic conditions. The control of the pH is important for effecting clean reductive amination. The pH range needed for the reduction of ketones and aldehydes is usually 3-4, whereas that for iminium cations is about 6-7. $NaBH_3CN$ works well within this pH range without causing unnecessary side reactions. However this method suffers for the formation of highly toxic byproducts as HCN and NaCN. $NaBH(OAc)_3$ and 2-picolineborane are used for the same purpose. Both have advantages in that the former has low toxicity and the latter can be used in water but their use is expensive. Other numerous alternative reagents such as tin hydrides, sodium borohydride, etc. have been reported, but some of them are not suitable to use in large scale production or are less selective[17]. Moreover, the presence of some metals in the final products and/or in the effluent of industrial processes can be a problem for human health and for the environment (see Table 1.10 in the Chapter 1). It is therefore essential to choose reaction conditions that have less impact on the environment. Reductive amination procedures by using Fe-triflate and $NaBH_4$ was recently reported[17]. It is important to note that, in comparison to other metals, Fe is recommended due to its non-toxic nature. However, although the use of Fe-triflate may seem a good alternative to traditional methods, it should be noted that this type of catalysts is not always recoverable and recyclable. For this reason, in the present research Aquivion®-Fe was tried as catalyst in this reaction. In fact its structure is a sort of a "metallic triflate polymer" but it has the great advantage of being easily removable from reaction mixture and it is potentially recyclable.

Mechanistically, iron triflate, as a Lewis Acid (LA), activates the carbonyl functionality and provide very reactive electrophile source. Amine that is used as substrate reacts with the activated aldehyde to afford hemiaminol equivalent. Thereafter, dehydration event regenerates the catalyst. In situ generated imine can further be reduced with sodium borohydride affording the products as shown in Scheme 3.12.9:



Scheme 3.12.9. Possible mechanism for direct amination

The reaction between aniline (**XXVIII**) and benzaldehyde (**LXXVII**) was investigated as model reaction in the presence (run 1, Table 3.12.4) and in absence of the catalyst (run 2, Table 3.12.4).



Scheme 3.12.10. Reductive amination between XVIII and LXXVII

The obtained results are reported in the following Table 3.12.4.

Table 3.12.4. Reductive amination reaction carried out in presence or in absence of Aquivion®-Fe

Run	Conv. LXVII (%)	XCV (%)	XCVI (%)	LXXVIII (%)	XCV isolated yield (%)[c]
1	99	80	6	13	60
2[a]	99	38	8	53	n.d
3[b]	99	90	---*	9	n.d

Reaction conditions: substrate **XXVIII** = 0.88 g (0.0094 mol); substrate **LXXVII** = 1 g (0.0094 mol); $NaBH_4$ = 0.35 g (0.0094 mol); of Aquivion®-Fe = 0.82 g (substrate (**XXVIII=LXXVII**)/-$R(SO_3)_3Fe$ ratio 32/1); dichlorometane = 15 mL; 3 mL of methanol were added after 3 h; T = 30°C; [a] in absence of catalyst; [b] substrate **XXVIII** = 0.088 g (0.00094 mol), substrate **LXXVII** = 0.1 g (0.00094 mol); CH_2Cl_2 = 3 mL; *after 10 minutes 90 % conversion into imine **XCVI** was observed than 0.035 g (0.00094 mol) of $NaBH_4$ and 1 mL of CH_3OH were added and after further 10 minutes a quantitative conversion of **XCVI** into **XCV** was detected; [c] after work-up.

The aldehyde and catalyst were added into the reactor in the same time and after 10 minutes the amine and $NaBH_4$ were added. It was observed that when the catalyst was used, the formation of intermediate imine **XCVI** is practically instantaneous but the formation of **XCV** is difficult. As soon as the methanol is added the amine **XCV** is formed. However, it was observed that the reaction between **LXXVII** and **XXVIII** is not complete, in fact, after 3 hours 13 % of unreacted **LXXVII** was checked; it was immediately converted into **LXXVIII** when the methanol was added. The work-up of crude mixture was simple: after reaction, it was washed with water and the catalyst was filtered off. The organic phase was separated from aqueous phase and the latter phase was extracted with dichlorometane. The organic phase was collected, the solvent was removed and after distillation under vacuum the desired product was obtained.

Working in absence of catalyst (run 2, Table 3.12.4), but adopting the previous described procedure, it was observed that the formation of imine intermediate **XCVI** is lower than when Aquivion®-Fe is used. After 3 hours, 58 % of unreacted **LXXVII** was determined and when methanol was added, the immediately formation of **LXXVIII** was observed.

From the obtained results, a catalyst role of Aquivion®-Fe, especially as regards the selectivity (reduced formation of **LXXVIII**), was observed in the reaction carried out under same conditions (runs 1 and 2, Table 3.12.4). However, it is also important to remark that when the reaction was carried out under different conditions ($NaBH_4$ was added after the intermediate imine **XCVI** was product, the conditions are reported in the experimental part, paragraph 5.15.10.8) a satisfactory result was obtained also in absence of catalyst.

It also important to note that, when the addition of methanol was made when the reaction was just started (test not reported in Table 3.12.4) the formation of desired amine **XCV** was not determined, but only **LXXVIII** formation was found. This means that the addition of methanol is fundamental and it should be made only when the imine **XCVI** is formed, because the reduction of aldehyde is easier than reduction of C=N double bond.

These results are confirmed by the literature data[17] where Iron-triflate was used. In the article it is reported that when the reaction is carried out without catalyst the reaction is incomplete and a relevant amount of alcohol is observed as by-product.

At the light of obtained results, the role of Aquivion®-Fe is still to be studied to define better benefits in terms of selectivity and activity of the reaction with different substrates. Nowadays, preliminary results obtained in research lab where this thesis has been developed, by using aliphatic aldehyde and benzylic amines, supports the hypothesis that Aquivion®-Fe could improve the selectivity of reaction[201].

Then, the study of this reaction catalyzed by Aquivion®-Fe should be focused in the future on these points:

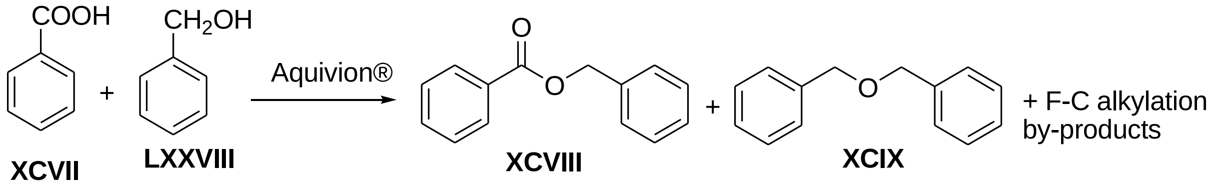
- careful study of reaction conditions in order to verify if the presence of catalyst is necessary or not;
- applications to other substrates in order to obtain products of industrial interest, especially verifying if the electronic and steric properties of used substrates could affect the outcome of the reaction;
- if the beneficial role of the catalyst was confirmed, the improvement of substrate/catalyst ratio to reduce the amount of catalyst and the possibility of recycling should be verified.

3.12.2.6 Synthesis of esters and ethers

The conventional esterification process involves the use of homogeneous acid system such as sulfuric acid or methane sulfonic acid. These acids are cheap but sometimes their use can lead to a sulfur contamination in ester compounds[185].

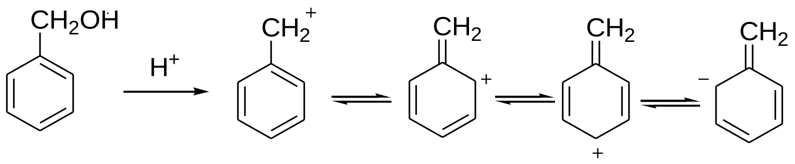
The potential use of Aquivion® as Brønsted solid recoverable and recyclable solid acid was initially investigated in the esterification of benzoic acid (**XCVII**) with benzyl alcohol (**LXXVIII**) in order to obtain benzyl benzoate (**XCVIII**), widely used as acaricide, scabicide, pediculicide and as repellent for mosquitoes chiggers and ticks.

The reaction was carried out at 80°C using a substrates **XCVII**/**LXXVIII**/-RSO_3H ratio 20/1 and **XCVII**/**LXXVIII** ratio 1:1 in toluene:



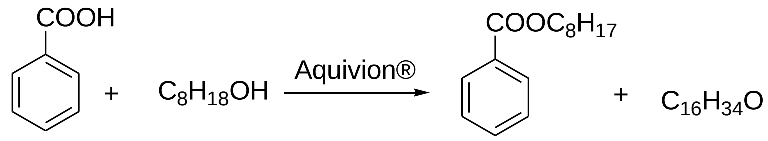
Scheme 3.12.11. Esterification reaction between XCVII and LXXVIII

After 4 hours, the full conversion of alcohol but not that of the acid was observed. The reaction resulted not particularly selective: besides the desired ester **XCVIII** (20 %), the formation of 1-((benzyloxy)methyl)benzene (**XCIX**) (48 %) and many Friedel-Crafts alkylation by-products was determined (31%). These results could be explained by the stability of benzyl carbocation involved in this reaction:



Scheme 3.12.12. Stability for resonance of the benzyl carbocation

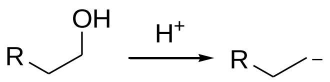
At the light of this obtained result, the esterification reaction was tried using octan-1-ol (**C**):



XCVII **C** **CI** **CII**

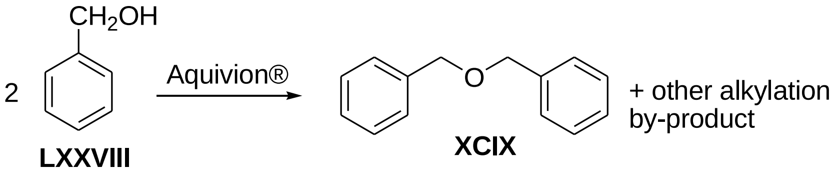
Scheme 3.12.13. Esterification reaction between XCVII and C

The reaction was carried out for 24 h at 80°C in dichloroethane by using substrate/-RSO$_3$H ratio 10/1. The conversion of **C** was 70 %. As expected , due to the non-stability of the aliphatic carbocation, in this case, the reaction was highly selective toward octyl ester **CI** (used GC analytical method however does not permit to exclude any rearrangement of the alkyl chain) that was obtained with 68 % chromatographic yield. Only 2 % of dioctyl ether **CII** as by-product was found and no Friedel-Crafts alkylation derivatives were observed.



Scheme 3.12.14. Linear carbocations does not have the resonance forms

In all previous examples, the presence of ethers as reaction by-products was detected. The formation of these compounds is already reported using heterogeneous acid such as Nafion®[202] or Amberlyst-15®, Dowex® M32[185]. At the light of these considerations, Aquivion® was also tried as catalyst in ether synthesis. Initially, the synthesis of 1-((benzyloxy)methyl)benzene (**XCIX**) was investigated:



Scheme 3.12.15. Etherification of LXXVIII

The reaction was carried out at 80°C for 3 hours in isooctane with a substrate **LXXVIII**/-SO_3H ratio 10/1. After 3 hours the conversion into **XCIX** was 65 % and after work-up the obtained yield was 40 %. In addition to desired product, the formation of alkylation by-product was detected due, also in this case, to the formation of a stable carbocation.

On the contrary, using octan-1-ol (**C**) as reagent and performing the reaction at 80°C for 24 h in isoctane with a substrate **C**/-RSO_3H ratio 10/1, only 6 % of desired product **CII** was obtained.

$$\underset{\textbf{C}}{2\ CH_3(CH_2)_7OH} \xrightarrow{\text{Aquivion®}} \underset{\textbf{CII}}{C_{16}H_{34}O} + H_2O$$

Scheme 3.12.16. Etherification of C

On the basis of the present results the application of Aquivion® as catalyst in these reaction does not seem very useful.

3.12.2.7 Reaction of amidation

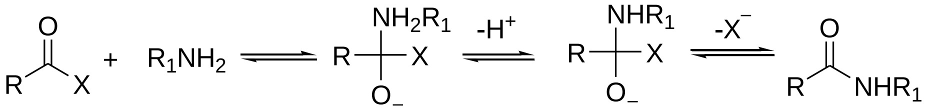
Another application of Aquivion® as catalyst was attempted in the direct synthesis of amide by the reaction between a carboxylic acid and an amine. This reaction is not easy because the equilibrium reaction is normally shifted towards the reactants:

$$\text{R-COOH} + R_1NH_2 \rightleftharpoons \text{R-COO}^- + R_1\overset{+}{N}H_3 \rightleftharpoons \text{R-CONHR}_1 + H_2O$$

Scheme 3.12.17. Equilibrium of amidation reaction

It is possible obtain a good yield by working under particular conditions such as bubbling the amine (or NH_3) in molten acid or by removing water by azeotropic distillation. It is to note that the amides may be also synthesized in the latter case also without any catalyst by simply heating at high temperature, if the thermal stability of the reagents allows high temperature. In this case, to improve the yield to amine, it is however necessary have an efficient method to remove the water so produced[203].

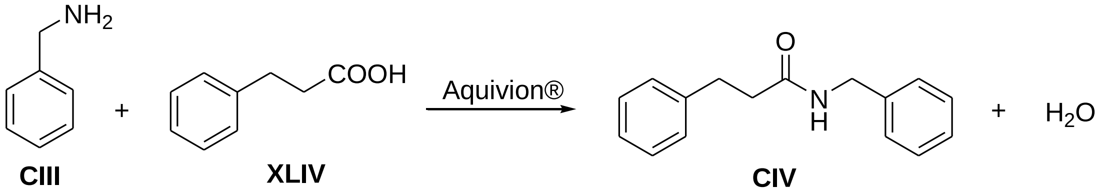
The most widely used method to synthetize an amide is the reaction between ammonia or primary or secondary amine with ester, anhydride or acyl halides:



Scheme 3.12.18. Amidation by using acid derivatives

When X is halogen or R-CO-O (but with less problems) the HX formation is an inconvenient because the acid can protonate the amine (or ammonia) reducing its nucleophilic behavior. If the amine is cheap, the excess of it might be used to force the reaction toward the products. Alternatively, a pyridine derivative or a tertiary amine or an inorganic base may be used to eliminate the hydrohalic acid (Schotten-Baumann reaction conditions). The amides can be also obtained from partial hydrolysis of nitrile operating by in 80 % solution of sulfuric acid or in presence of alkali and H_2O_2 or by Beckmann transposition (reaction between oximes and PCl_5 or H_2SO_4 etc) or by Ritter reaction (reaction between olefin or alcanol with nitrile in sulfuric acid).

The good-high yield may be reached with one of these indirect methods but a lot of wastes are usually produced. Therefore the direct synthesis of amides is an interesting challenge[204]. The use of Aquivion® as catalyst was tried on a model reaction with the aim to use milder condition and minimize wastes. In fact, the catalyst is solid, is simple to remove from the crude reaction mixture and is potentially recyclable. The first experiment was carried out between benzylamine (**CIII**) and 3-phenylpropionic acid (**XLIV**) at 110°C with substrates (**CIII=XLIV**)/-RSO_3H ratio 10/1 in toluene for 16 hours, in a simple reaction vessel without any equipment to remove water (Scheme 3.12.19).



Scheme 3.12.19. amidation reaction between CIII and XLIV

The formation of desired amide was observed (quantitative conversion of **CIII** intro desired product **CIV**, isolated yield 85 %), but the same result was surprising obtained, in the same conditions even when Aquivion® was not used (quantitative conversion **CIII** intro desired product **CIV**, isolated yield 82 %). This means that the amide in these conditions is formed by thermal effect and not thanks to the activity of catalyst (water probably was removed in the refrigerator). Therefore another test, using diisopropilamine and 3-phenylpropionic acid **XLIV** and Aquivion® as catalyst, was attempted working at different temperatures. The effect of temperature was studied by varying it from 40°C to 80°C. In all cases, no conversion to amide was detected after 20 h of reaction.

Finally at the light of a possible interaction of Aquivion®-Fe with the carbonyl group, this catalyst was also tried to synthetize amide directly. The reaction was carried out at 60°C in toluene for 20 h by using substrate (**CIII**=**XLIV**)/-$R(SO_3)_3Fe$ ratio 32/1 between benzylamine **CIII** and 3-phenylpropionic **XLIV** acid but after 24 hours only 6 % of desired amide was formed. In this case a modest catalytic effect was detected because at this temperature thermal reaction does not occur. Further experimental work with catalytic Aquivion®-Me could be tried even if this research seems to have a low probability of success.

4.CONCLUSIONS

This work of thesis had several targets to be achieved. Many of them are here summarized:

1. verification of the reproducibility of the procedure reported in a patent to obtain a very active heterogeneous catalyst with low content of rhodium by deposition of metal on a γ-alumina;
2. characterization of this Rh catalyst that had not been made previously;
3. checking the use of this Rh catalyst not only in the described hydrogenation of an α-β unsaturated ketone but also in other rhodium-catalyzed reactions; investigation on the versatility, activity, selectivity and recyclability of this catalyst;
4. verification of the potential versatility of this strategy for the preparation of other low metal content catalysts, based on Palladium and Platinum, checking their applicability, activity, selectivity and recyclability in different types of reactions and comparing their performances with those of some commercial catalysts;
5. preparation of new bio-generated metal species and their characterization in order to use them as new catalysts in different reactions;
6. preparation of new water soluble Rh-species, by using a cheap ligand such as dihydrothioctic acid salt, and test its potential activity in some Rh catalyzed reactions;
7. investigation on the potential applicability of a perfluorinated ionomer (Aquivion®) and some of its new metallic salts as Brønsted or Lewis Acid respectively;
8. preparation and purification of a non-commercial enzyme (CAR: carboxyreductase) as preliminary step for checking the possibility to extend its use into the reduction of a some not yet investigated carboxylic acid into the corresponding aldehyde;
9. investigation on the synthesis of some industrial targets characterized by the use of new homemade catalysts.

Below the more relevant results obtained during this research activity are reported.

As concerning targets 1-3

The reproducibility of patent procedure[35] to obtain a low 0.18 % Rh content catalyst has been confirmed. This procedure appears simple and easy to realize. The catalyst is stable and active for months. A detailed study, previously not made, on this heterogeneous catalyst, using different analytical techniques, such as atomic absorption, BET, X-ray, NH_3, CO and CO_2 chemisorption and TEM, has permitted to have a better knowledge of it, either as fresh material or after use, and to understand its performances as catalyst in a more depth. This homemade 0.18 % Rh/Al_2O_3 catalyst was used with success in many different Rh-catalyzed reactions as summarized below (Table 4.1).

Table 4.1. Different applications of homemade 0.18 % Rh/Al_2O_3 catalyst

Reaction	Aim	Results	Comments
Synthesis of Nabumetone (II), by hydrogenation of the α,β unsaturated precursor (I)	Verification of the possibility to replicate the procedure found in the patent[35], and attempt to recycle the catalyst by simple lab filtration procedure	The catalyst has showed high activity and selectivity toward the C=C double by operating under milder conditions (50°C, 0.5 MPa of H_2) and high ratio substrate **I**/Rh molar ratio (3700-5000). The catalyst was also easily recycled; using CO chemisorption analysis a partial aggregation of Rh particles was determined in the recycled material.	The aim has been reached, however an optimization of recycling procedure might be further developed.
Hydroformylation of styrene (IV)	Verification of the activity of 0.18 % Rh/Al_2O_3 in some other Rh-catalyzed reactions	The catalyst has showed a high activity and selectivity on hydroformylation of styrene (**IV**) by operating at 2 MPa of syngas and 50 °C and using a substrate **IV**/Rh molar ratio 800/1. Analysis of recovered catalyst showed a partial aggregation of Rh-particle and deposition of organic material. The catalyst was recycled with success.	In literature few example of application of Rh heterogeneous catalysts in hydroformylation reaction were previously known. The possibility to recycling, the low metal content and the good activity are the strengths of this catalyst. As future research activities: improvement of recovery procedure and extend its use toward other olefins to check its versatility .
Hydrogenation of phenol (VIII)	Verification of the activity of 0.18 % Rh/Al_2O_3 in some other Rh-catalyzed reactions	The catalyst has showed a good activity and fine selectivity toward the desired cyclohexanone (**IX**) under milder conditions (60°C, 0.5 MPa of H_2, with a substrate **VIII**/Rh molar ratio 1000/1) and performing the reaction in water.	The results are promising but further investigation is required to have a fine tuning of the reaction parameters, to improve the selectivity and to maintain a good activity of the catalyst in more recycles.
Hydrogenation of C=C double bond of trans-cinnamaldehyde (XXXIX)	Verification of the activity of 0.18 % Rh/Al_2O_3 in some other Rh-catalyzed reactions	The catalyst has showed a good activity and fine selectivity toward the C=C double bond under milder conditions (S/C: 1000-3800/1; $p(H_2) = 5$ MPa, 50°C. The formation of acetals was observed when the reaction was performed in ethanol	The preliminary results are encouraging but the reaction conditions will have to be further investigated in order to improve the substrate **XXXIX**/Rh molar ratio and the selectivity of reaction.
Preparation new fragrances by hydroformylation	Verification of the activity of 0.18 % Rh/Al_2O_3 in some other Rh-catalyzed reactions	The catalyst showed a good-high activity toward olefin with thienyl group in order to obtain new potential fragrances	The preliminary results are encouraging. It is to verify the possibility of recycling and to improve the reaction conditions.

As concerning targets 4

Another important obtained result was the preparation of two new different low metal content catalysts by using the same procedure. The attention was focused on production of new 0.28 % Pd/Al_2O_3 and recently of a new 0.27 % Pt/Al_2O_3 catalyst. Due to the low metal content, a deep characterization of 0.28 % Pd/Al_2O_3 was not possible until now. However, this new catalyst was successfully used in different types of reactions, showing very high versatility and selectivity.

Table 4.2. Different applications of homemade 0.28 % Pd/Al_2O_3 catalyst

Reaction	Aim	Results	Comments
Syntesis of β-ketoesters, in particular on haloaryl substrates, having agrochemical industrial interest	Try to use this new heterogeneous catalyst in the carboxymethylation reaction in absence of phosphine as Pd stabilizer.	The catalyst has showed good activity and selectivity compared to commercial heterogeneous and homogeneous catalysts. The recycling is possible.	In literature, this reaction was performed usually in the presence of homogenous catalysts containing phosphine ligands. During this work of thesis a good heterogeneous catalyst as alternative has been found. Further studies could be done to improve reaction conditions for scaling-up and fot application on other substrates.
Hydrodechlorination of haloaryl substrates	Possible application in the field of environmental remediation	The hydrodechlorination of 1,2,4 trichlorobenzene (**XXI**) as model substrate is complete by working under milder conditions (30°C, 0.2 MPa of H_2) in water in the presence of a base. The recycling was not possible	The completely deactivation of catalyst after first use could be a major drawback. Further studies should be made to understand the causes and find possible remedies. However it seems that Pd leaching is not a problem.
Reduction of aryl nitro groups	Possible application in the production of aniline and its halo-derivatives	The reduction of the nitro group is generally performed under mild conditions ($p(H)_2$=0.1-0.5 MPa, T = 25-50 °C, sub/cat molar ratio 100/500). Aniline (**XXVIII**) is selectively obtained, but when the reaction is performed on some nitro halo-derivative, partial or total dehalogenation is observed.	The completely dehalogenation and deactivation of catalyst towards nitro group when the 1-iodo-4-nitrobenzene (**XXXIII**) was studied, are the major drawbacks. Further studies should be made to understand the causes and find possible remedies. However, a good increase of selectivity was observed when the reaction was performed on 1-chloro-3-nitrobenzene (**XXXIV**) toward 3-chloroaniline (**XXXV**).
Reduction of C=C double bond of trans-cinnamaldehyde (XXXIX)	Verification of the selectivity of this catalyst	High selectivity toward reduction of C=C double bond is found working under milder conditions ($p(H)_2$ = 0.2-2 MPa, 35-60°C, sub/cat molar ratio 2500-32000). The activity is absolutely comparable or improved *vs* commercial catalysts prepared in similar manner. Formation of acetals is observed when the reaction is performed in alcoholic solvents, probably being this side reaction catalyzed by the support.	The obtained performances are very interesting for a possible industrial development.
Synthesis of aldehyde by reductive carbonylation from 2-ethyl-5-iodothiophene (LIV$_b$)	Verification the possibility to synthetize 5-ethylthiophene-2-carbaldehyde(**LVIII$_b$**), an industrial flavor, with a heterogenous catalyst instead of Pd-based homogeneous catalyst.	The reaction is performed under 5 MPa of CO, with substrate (**LIV$_b$**) /Pd molar ratio 50/1 by using PMHS as H-donor. A fine conversion of substrate into desired product is detected.	This result could be a good starting point to explore in details the use of heterogeneous catalysts in the reductive carbonylation.
Hydrogenation of phenol (VIII)	Check of the potential use of 0.28 % Pd/Al_2O_3 catalyst in the selective synthesis of cyclohexanone	Operating under milder conditions (60°C, 0.5 MPa of H_2, substrate/Pd molar ratio 1000/1) only 26 % conversion of phenol into desired product is detected.	A deeper study of reaction conditions should be made to overcome these disappointing results.

The new 0.27 % Pt/Al_2O_3 catalyst was analyzed for the moment only by CO chemisorption and analysis showed the presence of Pt-CO species. Currently, the catalyst was preliminarily tested only in the reduction of nitro group of 1-chloro-3-nitrobenzene (**XXXIV**) [p(H_2) = 0.5 MPa, T = 50°C, **XXXIV**/catalyst molar ratio 100/1)] and promising results were obtained. As matter of fact, no dechlorination was observed.

At the light of the obtained results, these new low metal content heterogeneous Pd- and Pt based catalysts showed a broad versatility. Furthermore it was possible to use them with success in the synthesis of intermediates and final products of the chemical industry. The big advantages of these catalysts are the easy preparation, the high activity and selectivity, the possibility of working under mild condition, the fine-good possibility of recycling and, last but not least, the low precious metal content which makes them cheap.

As concerning target 5

A new bio-generated metal species were directly obtained from fermentation of *Klebsiella oxytoca* BAS-10 in presence of Pd, Fe and Rh salts salt. During this work of thesis six new biogenerated species (1.7 % $(Pd\text{-}EPS)_{aerob}$, activated 1.7 % $(Pd\text{-}EPS)_{aerob}$, 13 % $(Pd\text{-}EPS)_{anaerob}$, 8.4 % Pd-Fe-EPS, 2,2 % Pd-Fe-EPS and 1.2 % Rh-EPS) were prepared that have shown a potential interesting catalytic activity in different types of reactions. Furthermore, different analysis (XPS, TEM, FT-IR) have allowed to obtain preliminary information on their characteristics. These new species were used in different types of reaction summarized in the following Tables 4.3, 4.4 and 4.5.

Table 4.3. Different applications of 1.7 % $(Pd\text{-}EPS)_{aerob}$ and activated 1.7 % $(Pd\text{-}EPS)_{aerob}$

Reaction	Aim	Results	Comments
Hydrodechlorination (catalyzed by activated 1.7 % $(Pd\text{-}EPS)_{aerob}$)	Possible application in the field of environmental remediation	The catalyst has showed low activity in the reaction on 1,2,4 trichlorobenzene (**XXI**)	
Reduction of nitro group	Possible application in the production of aniline (**XXVIII**) and its halo-derivatives	1.7 % $Pd\text{-}(EPS)_{aerob}$ showed good activity and selectivity into the reduction of nitrobenzene (**XXVII**) but under drastic conditions (80°C, $p(H_2)$=3 MPa, 24 h). In the reduction 1-iodo-4-nitrobezene (**XXXIII**), despite the different operating conditions, both catalysts afforded only dehalogenation reaction.	As in the case of 0.28 % Pd/Al_2O_3, the completely dehalogenation and the deactivation of catalyst towards the reduction of nitro group in 1-iodo-4-nitrobenzene (**XXXIII**), are the major drawbacks.
Reduction of αβ unsaturated C=C double bonds; preparation of Lioral^{TM} (LXI_a) and Helional^{TM} (LXIX) by hydrogenation of suitable precursors	Verification of selectivity of these species toward C=C double bonds; use in the preparation of commercial fragrances	The aqueous biphasic hydrogenation of α-β-unsaturated aldehydes has showed high selectivity towards C=C double bond. Activated 1.7 % $(Pd\text{-}EPS)_{aerob}$ is the preferred catalyst. Excellent results were obtained in the synthesis of Helional^{TM} (**LXIX**) and Lioral^{TM} (**LXI_a**) by hydrogenation of their unsaturated precursors. No enantioselectivity was observed.	Promising catalysts for the hydrogenation of other α-β-unsaturated compounds
Reductive carbonylation of 2-ethyl-5-iodothiophene (LIV_b) (catalyzed by 1.7 % activated $Pd\text{-}EPS)_{aerob}$	Check the possibility to synthetize 5-ethylthiophene-2-carbaldehyde (**$LVIII_b$**) under biphasic aqueous conditions	Fine activity and selectivity using TES as a hydrogen donor. Only dehalogenation reaction is observed when H_2 is used	The use of these catalyst could be a good alternative to conventional homogeneous catalysts in this reaction

Table 4.4. Different applications of 13 % $(Pd\text{-}EPS)_{anaerob}$

Reaction	Aim	Results	Comments
Hydrodechlorination	Possible application in the field of environmental remediation	13 % $Pd(EPS)_{anaerob}$ has showed discrete activity in the hydrodechlorination of 1,2,4 trichlorobenzene (**XXI**) under biphasic conditions. No complete dehydrodechlorination is found but recycling of catalyst is possible.	Further studies should be made in order to improve the reaction conditions and results
Syntesis of β-ketoesters	Try to use this new heterogeneous catalyst in the carboxymethylation reaction in absence of phosphine as Pd stabilizer.	13 % $Pd(EPS)_{anaerob}$ has showed a low selectivity in this reaction	The necessary presence of water favors undesired reaction. This catalyst is not suitable for this kind of reaction.

Table 4.5 Different applications of 8.4 % PdFe-EPS and 2.2 % PdFe-EPS 13 %

Reaction	Aim	Results	Comments
Hydrodechlorination	Possible application in the field of environmental remediation	Bimetallic EPS species have showed superior activity *vs* PD-EPS in the hydrodechlorination of 1,2,4 trichlorbenzene (**XXI**). The recycling seems possible.	Further studies should be made in order to improve reaction conditions and results.
Reduction of nitro group (catalyzed by 8.4 % Pd-FeEPS)	Possible application in the production of halo-derivatives of aniline	As other catalysts used, the dehalogenation of 1-iodo-4-nitrobenzene (**XXXIII**) with exclusive formation of nitrobenzene (**XXVII**) is observed. When the reduction of 1-chloro-3 nitrobenzene (**XXXIV**) was performed, despite the drastic conditions, some reaction intermediates are present.	These catalysts don’t seem promising.

Finally 1.2 % Rh-EPS was used with success in the hydroformilation of styrene (**IV**) operating under 3 MPa of syngas and 80 °C and in the hydroformylation of some thienyl olefins **LXIV$_b$** and **LXV$_b$** at 3-4 MPa of syngas and 60-100°C, for 20 h, in order to obtain new potential fragrances. This biogenerated catalyst showed a good high activity towards different types of substrates with the possibility of recycling. Further studies should be made to verify its use on different different substrates and in other Rh-catalyzed reaction.

In general, it is important to note that the investigation of these new-biogenerated species allowed to highlight their strengths and weaknesses in some reactions of industrial interest and in pollution remediation. The obtained results can be considered the basis for future studies that may also relate to the preparation of new species of metal or bimetal-polysaccharides. Furthermore, it is important to emphasize the potential application of this microorganism to sequestrate metals from aqueous wastes, embedding them in a polysaccharide secreted by the cells and easily purified; it could have relevant impacts either for producing catalysts in a cheaper way or in the environmental remediation.

As concerning target 6

This new water soluble specie, obtained by using dihydrothioctic acid as ligant, showed a high catalytic activity when was investigated in the hydroformylation of styrene (**IV**). The reaction was performed at 80°C, 8 MPa of syngas (CO/H_2=1) for 18 h with substrate **IV**/Rh molar ratio 1000/1 and quantitative conversion of styrene (**IV**) was observed also in the third performed recycled. The ratio between branched **V** and linear **VI** aldehyde remained constant. Furthermore, the good stability of [Rh(DHTANa)] in water , under inert atmosphere, was verified for months.
At the light of obtained results, this very promising new water soluble Rh species seems surely worth of a more detailed investigation on different applications in pure water or under biphasic conditions.

As concerning target 7

The preparation of some Aquivion® salts (Fe, Ga, In) by direct reaction of the ionomer with these metals to obtain new potential solid Lewis acids is another innovative result of the present research. This protocol is surely more sustainable than the exchange of cations used until now with other perfluorinated sulfonic polymers. The use of Aquivion® and Aquivion®-Me as potential catalysts was investigated in different reaction and the main results are summarized in Table 4.6.

Table 4.6. Potential applications of Aquivion® and Aquivion®-Me as acid catalysts

Reaction	Aim	Result	Consideration
Acylation of 2-ethylthiophene (LXXIX)	Use of of Aquivion® and Aquivion®-Me form as a possible alternative to the classical Friedel Crafts C-acylation catalysts.	Excellent results are obtained when Aquivion® salts are used under neat conditions. Promising new thienyl fragrances are obtained with satisfactory yield and a simple process protocol. The recovering and recycling of catalyst, for example, Aquivion®-Fe, is possible at least for three times with same performances.	The salt forms of Aquivion®, appear a good alternative to the classical acylation catalysts, such as $SnCl_4$ or commercial heterogeneous acid zeolites. Further activities to verify their versatility on other heterocycles is in progress.
Acylation of anisole (LXXXII), thianisole (LXXXIII), toluene, phenol (VIII)		When the reaction is performed on anisole (**LXXXII**) and thioanisole (**LXXXIII**) low-medium conversion is detected; with toluene, no reaction occurs, while when the reaction is carried out on phenol (**VIII**) only O-acylation is observed.	Further studies are needed to understand better the performances of this catalyst in the acylation of some aromatic substrates, verifying a possible positive role of the used solvent.
Fries Rearrangement	Use of Aquivion®-Fe as a possible Lewis for Fries Rearrangement on O-acyloxyphenol	No rearrangement is observed, only deacylation of substrate to phenol (**VIII**)	Check further the reaction conditions to exclude any role of the possible amount of water adsorbed on the catalyst
Alkylation of thiophene (XCI)	Use of Aquivion®-Fe or Aquivion® as possible Alkylation catalyst	Alkylation of thiophene (**XCI**) is observed when the reaction was performed with terbutylchloride and tertbutanol, but no reaction is found when propan-2-ol and 2-chloro-propane are used as alkylating agents	Limited interest for the use of these catalysts in Friedel-Crafts alkylation
Reductive amination	Use of Aquivion®-Fe as catalyst	Preliminary results have showed a potential benefit when Aquivion®-Fe was used with $NaBH_4$ as reducing agent. The selectivity of the reaction is improved.	Further studies should be focused on the possibility of recycling the catalyst and on the fine tuning of this type of reaction.
Etherification and esterification	Use of Aquivion® as possible acid catalyst	Low selectivity was observed especially when the aromatic substrates were used.	Aquivion® does not seem to be advantageous *vs* other cheaper traditional systems.
Amidation		The formation of desired amide may occur also in absence of catalyst.	Aquivion® does not seem to be advantageous in the amidation reaction.

The obtained results are a good basis for the development and use of this ionomer, especially as iron and gallium salts, as new Lewis Acids. The simple preparation, the possibility of recycling and the possibility of working also in the absence of auxiliary organic solvent are the major strengths of these new Lewis acids species. However, further studies must still be done to test their versatility.

As concerning target 8

Not commercially available CAR enzyme, produced by fermentation of genetically modified microorganism, has showed a fine-good selectivity in the direct reduction of 3-phenylpropionic (**XLIV**) acid into 3-phenylpropanal (**VI**), an industrial aroma, working in water medium. However, the formation of undesired 3-phenylpropanol (**XL**) was observed when crude enzyme was used (CFE). To overcome this problem, the purification of the enzyme is mandatory and has been carried out during the present thesis. In general, the use of this enzyme seems to be promising as an alternative to traditional methods of reduction of carboxylic acids, even if many problems/drawbacks have to be solved, including the use of stoichiometric amount of co-factor $NADPH^+$, which must be recycled, the impossibility to reuse the free enzyme and the present commercial unavailability. For these reasons, further studies should be made in the next future. The present contribution is however a good starting point, that has showed also the potential application to the production of a “natural labeled” aroma.

As concerning target 9

The potential application as these homemade catalysts was verified not only on commercial substrates but also on substrates synthesized in the laboratory, where this thesis work was carried out. The attention, in particular, was focused on two fragrances Lioral^{TM} (**LXI$_a$**) and Helional^{TM} (**LXIX**). During the synthesis of these two products, also other interesting products were obtained such as 5-isopropylthiophen-2-carbaldehyde (**LVIII$_a$**), 5-ethylthiophen-2-carbaldehyde (**LVIII$_b$**), molecules structurally similar to Lioral^{TM} (**LXI$_a$**) and potentially usable as fragrances (**LXI$_b$, LXVII$_b$, LXVIII$_b$**). Furthermore, it was also obtained Piperonal (**LXXI**) which is an intermediate to obtain Helional^{TM} (**LXIX**) but also is an important industrial substrate.

It is however to underline the numerous difficulties to prepare some not easily available reagents/intermediates for the production of the above indicated active ingredients. It was necessary to overcome many obstacles and it was possible only through a deep analysis of the process and fine tuning of reaction parameters. Among the most important achieved results, it is to mention before achieving the desired fragrance: a) a satisfactory synthesis of 2-isopropyl-thiophene (**L$_a$**) key material for Lioral^{TM} **LXI$_a$**; b) improved methods of halogenation of thiophene rings; c) satisfactory reductive carbonylation, by homemade catalysts, to obtain thienylaldehydes;

d) high selective hydrogenation of unsaturated precursors of Lioral^{TM} **LXI$_a$** and pseudo Lioral **LXI$_b$** with a biogenerated homemade catalyst. Also in the case of Helional^{TM} **LXIX** improved methods of halogenation of methylenedioxybenzene, of the innovative reductive carbonylation to obtain Piperonal, of high selective hydrogenation of unsaturated precursor of Helional^{TM} **LXIX** were carried out to achieve finally the desired fragrance.

In conclusion this research work has not only achieved many relevant targets but also it should have identified solid starting points for further research activities finalized to potential industrial applications.

5. EXPERIMENTAL PARTS

5.1 Commercial reagents, materials and solvents

(E)-4-(2-methoxynaphthalen-6-yl)but-3-en-2-one (generous gift by Prof. Piccolo)

1,3[d]Benzodioxole (Sigma-Aldrich)

1-Bromoprop-1-ene (Sigma-Aldrich)

1-Chloro-3-nitrobenzene (Schuchardt Munchen)

1-Iodo-4-nitrobenzene (Sigma-Aldrich)

2-Acetylthiophene (Sigma-Aldrich)

2-Bromoprop-1-ene (Sigma-Aldrich)

2-Bromo-thiophene (Sigma-Aldrich)

2-Chloro-1-(2,4-dichlorophenyl)ethanone (Sigma-Aldrich)

2-Chloro-1-(4-chlorophenyl)ethanone (Isagro)

2-Chloro-1-phenylethanone (Isagro)

2-Ethyl-thiophene (Sigma-Aldrich)

3-Bromoprop-1-ene (Sigma-Aldrich)

3-Phenylpropanal (Sigma-Aldrich)

3-Phenylpropionic Acid (Sigma-Aldrich)

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES buffer) (Sigma-Aldrich)

Aniline (Sigma-Aldrich)

Benzaldehyde (Sigma-Aldrich)

Absolute acetic acid (Sigma-Aldrich)

Absolute Ethanol (VWR)

Acetic anhydride (Sigma-Aldrich)

Acetonitrile (Sigma-Aldrich)

Adenosine 5'-triphosphate disodium salt (Sigma-Aldrich)

Ammonium chloride (Carlo Erba)

Ampicillin (Sigma-Aldrich)

Anisole (Sigma-Aldrich)

Benzoic Acid (Sigma-Aldrich)

Benzophenone (Janseen Chemical)

Benzyl alcohol (Sigma-Aldrich)

Benzyl amime (Sigma-Aldrich)

Buffer pH 10 (boric acid, potassium chloride, sodium hydroxide) (Carlo Erba)

Butylhydroxytoluene (BHT) (Sigma-Aldrich)

Calcium Chloride (Fluka)

Carbon Monoxide (SIAD)

Chloramphenicol (Sigma-Aldrich)

Chloroform (VWR)

Copper sulfate pentahydrate (Carlo Erba)

Dichloromethane (VWR)

Diethylether (VWR)

Diisopropyl ether (Sigma-Aldrich)

Dimethyl sulphoxide (Carlo Erba)

Distilled water

Ethyl acetate (VWR)

Gallium (Sigma-Aldrich)

Glucose (Sigma-Aldrich)

Glycerol (Sigma-Aldrich)

Hexamethylphosphoramide (HMPA) (Merck)

Hydrochloric acid (Sigma-Aldrich)

Hydrogen (SIAD)

Hydrogen bromide 48 % (Sigma-Aldrich)

Hydrogen peroxide 33 % (Sigma-Aldrich)

Imidazole (Sigma-Aldrich)

Indium (Sigma-Aldrich)

Iodine (Sigma-Aldrich)

Iodine (Sigma-Aldrich)

Iron (gently furnished by Cambrex)

Iron citrate (Carlo Erba)

Isoctane (Sigma-Aldrich)

Lithium aluminum hydride (Sigma-Aldrich)

Magnesium (Janseen Chimica)

Magnesium chloride heptahydrate (Sigma-Aldrich)

Magnesium sulfate (Sigma-Aldrich)

Methyl magnesium bromide in diethyl ether solution (Sigma-Aldrich)

Methyl terbutyl ether (MTBE) (Sigma-Aldrich)

$NADP^+$ (sodium salt) (Sigma-Aldrich)

NADPH (tetrasodium salt) (Sigma-Aldrich)

N-Bromosuccinimmide (NBS) (Fluka)

n-Hexane (Sigma-Aldrich)

n-Hexane for HPLC (Sigma-Aldrich)

Nitric Acid (Sigma-aldrich)

Nitrobenzene (Carlo Erba)

N-methyl-formanilide (Sigma-Aldrich)

Octan-1-ol (Sigma-Aldrich)

Oxone® (Sigma-Aldrich)

Palladium dichloride (Sigma-Aldrich)

Palladium nitrate di-hydrate (Sigma-Aldrich)

Phenol (Fluka)

Phosphoryl trichloride (Sigma-Aldrich)

Polymethylhydrosiloxane (PMHS) (Sigma-Aldrich)

Potassium iodate (Janseen Chmica)

Potassium chloride (Sigma-Aldrich)

Propionaldehyde (Fluka)

Propionic anhydride (Sigma-Aldrich)

Propionyl chloride (Sigma-Aldrich)

Rhodium chloride (Sigma-Aldrich)

Rhodium nitrate (Sigma-Aldrich)

Silica (Sigma-Aldrich)

Sodium (Sigma-Aldrich)

Sodium borohydride (Carlo Erba)

Sodium Carbonate (Carlo Erba)

Sodium Chloride (Sigma-Aldrich)

Sodium Citrate (Sigma-Aldrich)

Sodium Formate (Sigma-Aldrich)

Sodium Benzoate (Sigma-Aldrich)

Sodium hydrogencarbonate (Carlo Erba)

Sodium Hydroxide (Carlo Erba)

Sodium hypochlorite 10-15 % (Sigma-Aldrich)

Sodium iodate (Sigma-Aldrich)

Sodium phosphate monobasic (Sigma-Aldrich)

Sodium sulphate (Sigma-Aldrich)

Sodium thiosulfate (RP Normapur Prolabo)

Sulfuric Acid 98 % (Sigma-Aldrich)

Terbutylchloride (Sigma-Aldrich)

Tert-butyl alcohol (Sigma-Aldrich)

Tetrahydrofuran (VWR)

Thioanisole (Sigma-Aldrich)

Thiophene (Sigma-Aldrich)

Thioctic acid

Tin tetrachloride (Sigma-Aldrich)

Toluene (VWR)

Triethylsilane (Fluorochem)

Trifluoroacetic acid (Sigma-Aldrich)

Trioctylamine (Sigma-Aldrich)

Water

5.2 Commercial catalysts and ligands

$[HRh(CO)(PPh_3)_3]$ (Strem Chemicals)

$[Pd(PPh_3)_2Cl_2]$ (Strem Chemicals)

3, 3′,3″-Phosphanetriyltris(benzenesulfonic acid) trisodium salt (Fluka)

5 % Pd/C (Chimet)

$Pd(OAc)_2$ (Sigma-Aldrich)

$Pd(PPh_3)_4$ (Sigma-Aldrich)

$PdCl_2$ (Sigma-Aldrich)

$Pd(NO_3)_2$ (Sigma-Aldrich)

$PtCl_2$ (Sigma-Aldrich)

$RhCl_3$ (Sigma-Aldrich)

DABP (diadamantyl butyl phosphine)

5.3 Commercial polymers and acid catalysts

Aquivion®(Solvay Specialty Polymers)

Amberlyst 15® (Room and Haas)

EPZ-10 (Contract chemicals)

5.4 Commercial culture media

LB Broth (Novagen)

Overnight Express (Novagen)

5.5 Specific commercial material used for the purification of the CAR enzyme

Cellulose acetate dialysis membrane (Sigma-Aldrich)

Nickel-Agarose solution (Qiagen)

Phenylmethanesulfonylfluoride (PMSF) (Sigma-Aldrich)

5.6 Purification of solvents

The purification of solvents was performed as reported in literature[205].

5.6.1 Dichloromethane

In a 1000 mL double neck bottom flask, 500 mL of dichloromethane and 50 g of $CaCl_2$ were introduced under nitrogen. The solution was mixed for 24 h, than was filtered under nitrogen and transferred into a 1000 mL double neck bottom flask, where 50 g of drierite were previously introduced. Then dichloromethane was warmed at reflux for 8 hours and subsequently recovered by distillation into a bottle containing molecular sieves.

5.6.2 Diethyl ether

In a 1000 mL three neck bottom flasks, 500 mL of commercial diethyl ether, 30 g of benzophenone and small pieces of metallic sodium were introduced under nitrogen. The solvent was warmed at reflux for 12 hours under nitrogen. After this time, diethyl ether was transferred by distillation into a bottle containing molecular sieves.

5.6.3 Tetrahydrofuran

In a 1000 mL three neck bottom flasks, 500 mL of commercial tetrahydrofuran, 30 g of benzophenone and small pieces of metallic sodium were introduced under nitrogen. The solvent was warmed at reflux for 12 hours under nitrogen. After this time, the solvent was transferred by distillation into a 1000 mL three neck bottom flasks containing $LiAlH_4$. Tetrahydrofuran was maintained at reflux for six hours. After this time, the solvent was transferred by distillation into a bottle containing molecular sieves.

5.7 New low metal content catalysts

0.18 % Rh/Al_2O_3

0.28 % Pd/Al_2O_3

0.27 % Pt/Al_2O_3

5.8 New biogenerated species

The new biogenerated species were obtained by fermentation of *Klebsiella oxytoca* BAS-10

1.7 % $(Pd\text{-}EPS)_{aerob}$

Activated 1.7 % $(Pd\text{-}EPS)_{aerob}$

13 % $(Pd\text{-}EPS)_{anaerob}$

2.2 % Pd-Fe-EPS

8.4 % Pd-Fe-EPS

1.2 % Rh-EPS

5.9 New water-soluble species

[Rh(DHTANa)]

5.10 Non-commercial enzyme prepared by fermentation

The enzyme was obtaneid by fermentation of *Escherichia coli* BL21 (DE3) Carboxyreductase (CAR Enzyme).

5.11 New metal Aquivion® compunds

Aquivion®-Fe

Aquvion®-Ga

Aquivion®-In

5.12 Main products prepared during this work of thesis

1-((benzyloxy)methyl)benzene (**XCI**)

1-(2-(methylthio)phenyl)ethanone (**LXXXV**)

1-(2,4-dichlorophenyl)ethanone (**XX**)

1-(2-methoxyphenyl)ethanone (**LXXXVI**)

1-(-3,3-diethoxyprop-1-enyl)benzene (**XLIII**)

1-(3,3-diethoxypropyl)benzene (**XLII**)

1-(4-(methylthio)phenyl)ethanone (**LXXXVII**)

1-(4-chlorophenyl)ethanone (**XX**)

1-(4-methoxyphenyl)ethanone (**LXXXIV**)

1-(5-ethylthiophen-2-yl)ethanone (**LXXXI**)

1-(5-ethylthiophen-2-yl)propan-1-one (**LXXX**)

1,2 dichlorobenzene (**XIII**)

1,2-bis(3-chlorophenyl)diazene (**XXXVII**)

1,2-diphenyldiazene (**XXX**)

1,3 dichlorobenzene (**XIV**)

1,4 dichlorobenzene (**XV**)

2-(5-ethylthiophen-2-yl)butanal (**LXXVII$_b$**)

2-(prop-1-en-2-yl)thiophene (**LI**)

2-(thiophen-2-yl)propan-2-ol (**XLIX**)

2-allyl-5-ethylthiophene (**LXV$_b$**)

2-Bromo-5-ethylthiophene (**LV$_b$**)

2-Chloro-5-ethylthiophene (**LVI$_b$**)

2-ethyl-5-(prop-1-enyl)thiophene (**LXIV$_b$**)

2-Ethyl-5-iodothiophene (**LIV$_b$**)

2-hydroxy-1-phenylethanone (**XVI**)

2-Iodo-5-isopropylthiophene (**LIV$_a$**)

2-isopropyl-5-(propen-1-il)-thiophene (**LI**)

2-Isopropylthiophene (**L$_a$**)

2-Phenylpropanal (**V**)

2-terbutilthiophene (**XCI**)

2-Terbutylthiophene (**XCII**)

3-(5-ethylthiophen-2-yl)-2-methylpropanal (**LXI$_b$**)

3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (**LX$_a$**)

3-(5-isopropylthiophen-2-yl)-2-methylpropanal (Lioral^{TM}) (**LXI$_a$**)

3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (**LXXII**)

3-(benzo[d][1,3]dioxol-6-yl)-2-methylpropanal (Helional^{TM}) (**LXIX**)

3-chloroaniline (**XXXV**)

3-phenylpropan-1-ol (**XL**)

3-Phenylpropanal (**VI**)

3-terbutiltiophene (**XCII**)

3-Terbutylthiophene (**XCIII**)

4-(2-methoxynaphthalen-6-yl)butan-2-one (**II**)

4-(5-ethylthiophen-2-yl)butanal (**LXVIII$_b$**)

4-iodoaniline (**XXXIII**)

5-Bromobenzo[d][1,3]dioxole (**LXXIV**)

5-ethylthiophene-2-carbaldehyde (**LVIII$_b$**)

5-ethylthiophene-2-carboxylic acid (**LIX$_b$**)

5-iodobenzo[d][1,3]dioxole (**LXXIII**)

5-isopropylthiophene-2-carbaldehyde (**LVIII$_a$**)

Acetophenone (**XV**)

Aniline (**XXVIII**)

Benzene (**XII**)

benzo[d][1,3]dioxole-5-carbaldehyde (piperonal) (**LXXI**)

Benzylbenzoate (**XC**)

Chlorobenzene (**XIII**)

Cyclohexanol (**X**)

Cyclohexanone (**IX**)

Dioctylether (**CII**)

Methyl 3-(2,4-dichlorophenyl)-3-oxopropanoate (**XIII**)

Methyl 3-(4-chlorophenyl)-3-oxopropanoate (**XII**)

Methyl 3-oxo-3-phenylpropanoate (**XIV**)

N,N$'$ –bis(3-chlorophenyl)diazene n-oxide (**XXXVIII**)

N,N'-diphenyl-diazene N-oxide (**XXXI**)
N-benzyl-3-phenylpropanamide (**CIV**)
N-benzylbenzenamine (**XCV**)
N-benzylidenebenzenamine (**XCVI**)
Nitrosobenzene (**XXIX**)
Octyl benzoate (**CI**)

5.13 Analytical method

5.13.1 Nuclear magnetic resonance analysis

^{1}H-NMR spectra were recorder by Bruker Avance 300 operating at 300 MHz. The used solvents were deuterated chloroform, deuterated methanol and deuterated water.

5.13.2 Gas chromatographic analysis

GC analysis were performed on a Agilent Technologies 6850 series equipped by HP-5 (Agilent) capillary column (30m x 0.32mm x 0.25 μm film thickness) and flame ionizator detector (FID). The instrument is interfaced with a computer. The GC parameters were as follows: initial temperature 50°C; initial time 5 min; heating ramp 10°C/min; final temperature 280°C, final time 5 min; injector temperature 280°C; detector temperature 280°C, gas carrier flow (N_2) 2 mL/min, injection volume 0.1 μL.

5.13.3 GC-MS analysis

GC-MS analysis were performed on a ThermoFinnigan (Trace CG 2000) equipped with an HP-5 capillary column (30m x 0.32mm x 0.25 μm film thickness) and a quadrupole mass spectrometer (ThermoFinnigan Trace MS) interfaced with a computer. The GC parameters were as follows: initial temperature 60°C; initial time 5 min; heating ramp 10°C/min; final temperature 280°C, final time 5 min; injector temperature 280°C; detector temperature 280°C, gas carrier flow (N_2) 0.8 mL/min, injection volume 0.1 μL.

5.13.4 HPLC analysis

HPLC analysis was performed on a HP 1100 series equipped by Phenomenex Lux %u cellulose-1 (4.6 mm x 250 mm) column and a peristaltic pump. The flow of the eluent mixture n-hexane/isopropanol (99/1) was set at 1 mL/min.

5.13.5 Transmission electron microscope observations

To observe the electron dense palladium in EPS, several samples were prepared for transmission electron microscopy (TEM), before and after the pre-treatment of Pd-EPS with hydrogen. The treated and untreated dried catalyst was re-suspended in distilled water, and after 1 min, 10 μl of suspension were placed on a platinum grid treated with Formvar resin biofilm. The liquid was evaporated at room temperature and the samples were observed by TEM (JEOL JEM 100b microscope) operating under standard conditions.

5.13.6 X-ray photoelectron spectroscopy (XPS)

XPS data were obtained on an experimental apparatus in UHV consisting of a modified Omicron NanoTechnology MXPS system, with an XPS chamber equipped with a dual X-ray anode source (Omicron DAR 400) and an Omicron EA-127 energy analyzer, and an attached VT-atomic force and scanning tunneling microscope.

Sample transfer between the various experimental areas was conducted by means of linear magnetic transfer rods or manipulators. MgKa photons were used (hv = 1253.6 eV), generated operating the anode at 14–15 kV, 10–20 mA. Binding energy (BE) values were derived from experimentally determined kinetic energies (KEs) from the relation:

$$BE = h\nu - KE$$

The spectrometer energy was calibrated by metal standards. The samples were mounted as finely grounded powders on metal tips. No sizeable sign of sample degradation under the acquisition times under the X-rays was observed for the samples.

The theoretical reconstruction of the curves was made by using a series of commercially available software routines, always using Gaussian-Lorentzian mixed shapes. XPS atomic ratios were estimated from experimentally determined area ratios of the relevant core lines, corrected for the corresponding theoretical atomic cross-sections and for a square-root dependence of the photoelectrons kinetic energies. A Shirley-type background was subtracted from the experimental curves. Atomic ratios are associated with an error of ± 10%. The accuracy in reported BEs is ± 0.2 eV and the reproducibility of results is within these values. All reported spectra were acquired at a photoelectron take-off angle of 11° measured from the surface normal. As evidenced by a rigid energy shift of the overall spectrum, the NPs experienced static charging under X-rays to different amounts, which were quantified and corrected by taking the more intense C 1s peak component, likely due to the compounds, at 285.0 eV.

5.13.7 CO, CO_2 and NH_3 chemisorption analysis

For FT-IR characterization powders were pressed into thin self-supporting pellets and then placed into a quartz IR cell. In particular, prior to the adsorption measurements, all samples were activated by a treatment in H_2 (20 KPa) at 150°C (heating rate 2.5°C/min) for 1h, and then cooled to room temperature under Ar atmosphere. FT-IR spectra were collected by using a Bruker Equinox 55 spectrometer, equipped with MCT cryodetector, at a spectral resolution of 2 cm^{-1} and accumulation of 32 scans. The interaction with NH_3, CO_2 and CO was studied at room temperature in the relative pressure range 0.01-3 Pa.

5.13.8 X-ray analysis of Al_2O_3

The sample of Al_2O_3 was characterized by powder X-ray diffraction (XRD) through a Philips X'pert diffractometer (Cu Kα radiation) and nitrogen adsorption measurements at 77 K performed using a Quantachrome Autosorb 1.

5.13.9 BET analysis

BET specific surface areas of 0.18 % % Rh/Al_2O_3 and Al_2O_3 has been calculated in the relative pressure range 0.04–0.1 and pore size has been evaluated following the BJH method.

5.13.10 Atomic absorption measures

The sample of 0.28 % Pd/Al_2O_3, 0.27 % Pt/Al_2O_3 and all Me-EPS species after treatment with aqua regia, were analyzed by atomic absorption, by using a Perkin Elmer ANALYST 100, to determine the concentration of Pd, Fe and Pt.

5.13.11 Analysis of 1.2 % Rh-EPS and 0.18 % Rh/Al_2O_3

The analysis of Rh content was performed by Chemilab by adopting the UNI EN 13674:2004 + EPA 6010C 2007 protocol.

5.13.12 Analysis of Aquivion®-Fe and Aquivion®-Ga

In a closed vessel the samples (50 mg) were suspended in a mixture of concentrated HNO_3 (65%, 6 mL) and concentrated HCl (37%, 2 mL). The vessel was pressurized with N_2 till reaching a pressure of 40 atm and then heated at 250°C for 45 min. The clear and transparent liquid was recovered and then metal content determined through ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). The analysis was repeated twice.

5.13.13 IR-analysis

The IR spectra of [Rh(DHTANa)], 1.7 % $(\text{Pd-EPS})_{\text{aerob}}$ and Na-EPS were record by FTIR Nicolet Magna 750 instrument. The samples were prepared on KBr pellets.

5.13.14 SDS-Page Analysis

Cell free extracts (CFE) and pure enzyme CAR were analyzed by SDS-PAGE electrophoretic analysis. The samples were treated with mercaptoethanol and water, and deposed on polyacrylamide gel.

The analysis was performed at 165 volts for 35 minutes by using tris-acetate buffer. After this time, the gel was treated with ISTANTblue® and analyzed.

5.14 Preparation of new metal catalysts

5.14.1 Preparation of homemade 0.18 % Rh/Al_2O_3, 0.28 % Pd/Al_2O_3, 0.27 % Pt/Al_2O_3

All homemade catalysts were prepared by adopting the procedure below reported. For example the procedure to obtain 0.18 % Rh/Al_2O_3 catalyst is reported

50 mg of $RhCl_3$ (0.00023 mol of Rh), 0.36 mL (0.0008 mol) of TOA (d = 0.809 g/mL) and 10 mL of dry THF were introduced in a 150 mL stainless steel autoclave under nitrogen. The autoclave was pressurized with 0.5 Mpa of hydrogen and warmed at 25 °C for 24 hours under stirring. After this time, the solution was introduced into a 250 mL double neck jacket round bottom flask containing 10 g of alumina and 30 mL of dry THF. The mixture was stirred for 24 h at 25°C under hydrogen atmosphere. After this time, the solid was filtered by synthered glass filter and washed with 50 mL of dry THF. The recovered catalyst was dried under vacuum at 0.06 MPa. After atomic absorption the detected amount of Rh was 0.18 %.

Adopting the same procedure a homemade Pd/Al_2O_3 and Pt/Al_2O_3 catalyst were prepared by using:

50 mg of $PdCl_2$ (0.00028 mol of Pd), 0.44 mL (0.00098 mol) of TOA (d = 0.809 g/mL) and 10 g of Al_2O_3. After atomic absorption, the detected amount of Pd was 0.28 %.

41 mg of $PtCl_2$ (0.00015 mol of Pt),0.24 mL (0.00054 mol) of TOA and 10 g of Al_2O_3 respectively. After atomic absorption, the detected amount of Pt was 0.27 %.

Furthermore, a 1 % Pd/acid Al_2O_3 was also prepared but the amount of Pd was not detected.

5.14.1.1 Preparation of 1 % Pd/Acid Coal

The preparation of this homemade catalyst was carried out by adopting the procedure reported in literature[206].

In a round a 250 mL round bottom flask equipped with magnetic stirrer, 10 g of Acid Coal superwj, 0.21 g of $Pd(OAc)_2$, and 25 mL of THF were added. The mixture was stirred under hydrogen atmosphere for 24 h. Subsequently, THF was removed by distillation under vacuum. The amount of Pd was not determined.

5.14.2 Preparation of new bio-generated metal species

5.14.2.1 Preparation of 1.7 % (Pd-EPS)$_{aerob}$

1.7 % Pd-EPS was obtained by aerobic cultivation of *Klebsiella oxytoca* BAS-10 in static mode. *Klebsiella oxytoca* BAS-10 was retrieved from cryovials kept at −80 ◦C in 25% glycerol in Nutrient broth (Difco). An aliquot of 1 ml of overnight culture of BAS-10 was transferred in a media containing per liter: 2.5 g $NaHCO_3$, 1.5 g NH_4Cl, 1.5 g $MgSO_4 \cdot 7H_2O$, 0.6 g NaH_2PO_4, 0.1 g KCl, and 50 mM (14.7 g/L) Na-citrate, hereafter referred to as NAC medium, was buffered at pH 7.6 with NaOH. The culture was inoculated (1:100, v:v) and incubated at 30°C. At the beginning of the stationary growth phase (2 days, optical density > 1 at 600 nm) in static mode the BAS-10 culture was amended with 50 mg/L of Pd as of $Pd(NO_3)_2$ (108 mg) and shaken with magnetic bar for 24 h. After this time, bacterial suspensions of *Klebsiella oxytoca* were resupended in fresh medium and centrifuged to eliminate bacterial cells and then the supernatant was treated with 800 mL of cooled ethyl alcohol (95%) to precipitate the metal polysaccharide. Salt residues were removed by washing Pd-EPS three times with distilled water. The washing was repeated twice. The colloidal material was dried out under vacuum to obtain Pd-EPS, as solid material, that was stored at 4°C.

5.14.2.2 Activated 1.7 % Pd-EPS preparation

A suspension of Pd-EPS (100 mg) in 20 mL of distilled water was stirred at 30°C and 1 MPa of hydrogen for 21 h. Water was then distilled off and the gray solid obtained dried under vacuum.

5.14.2.3 Preparation of 13 % Pd-EPS

13 % Pd-EPS was obtained by cultivation of *Klebsiella oxytoca* BAS-10 in anaerobic conditions. The cells were inoculated (1:100 v:v) in 1 liter NAC medium by adopting the same procedure described at paragraph 5.14.2.1 previously autoclaved and cooled down under fluxing N_2, in sealed pyrex bottles. When the cell density (≈ 1.0 Abs 600 nm) was reached, the BAS-10 culture was amended

with 50 mg of Pd as $Pd(NO_3)_2$ (108 mg) under fluxing N_2. The pyrex bottle was sealed again and the culture was incubated for further 7 days at 30°C until the metal-enriched polysaccharide was precipitated in the bottom and then extracted from the cell culture by adopting the above describe procedure for 1.7 % Pd-EPS at paragraph 5.14.2.1

5.14.2.4 Preparation of 8.4 % Pd-Fe-EPS and 2.2 % Pd-Fe-EPS catalysts

The cultivation of strain BAS-10 was performed in 1 L of medium incubating at 30°C under anaerobic conditions but using two different media culture:

1. The composition of media culture to obtain 8.4 % Pd-Fe-EPS is: 2.5 g $NaHCO_3$, 1.5 g NH_4Cl, 0.6 g, 1.5 g $MgSO_4.{\cdot}7H_2O$, 0.6 g NaH_2PO_4, 0.1 g KCl, 14.3 g of Na-citrate and 0.235 g of Fe (III)-citrate (50 mg/L as Fe). 8.4 % Pd-Fe-EPS was obtained from this media culture.
2. The composition of media culture to obtain 2.2 % Pd-Fe-EPS catalyst is: 2.5 g $NaHCO_3$, 1.5 g NH_4Cl, 0.6 g, 1.5 g $MgSO_4{\cdot}7H_2O$, 0.6 g NaH_2PO_4, 0.1 g KCl, 13.23 g Na-citrate and 1.315 g Fe(III)-citrate (280 mg/L as Fe). 2.2 % Pd-Fe-EPS was obtained from this media culture.

When a good cell density (≈ 1.0 Abs 600 nm) was reached in the two media, each culture was amended with 50 mg of total Pd as $Pd(NO_3)_2$ (108 mg). The anaerobic conditions were maintained by fluxing N_2 until the culture and the pirex-bottles were sealed again and incubated for further 7 days. At the end of this time, the 8.4 % Pd-Fe-EPS and 2.2 % Pd-Fe-EPS were recovered by adopting the same procedure reported at paragraph 5.14.2.1 for 1.7 % Pd-EPS. From 1 L of culture 0.45 g of 8.4 % Pd-FE-EPS and 1.1 g of Pd-Fe-EPS were recovered respectively. The amount of Pd and Fe were determined by Atomic absorption.

Table 5.1. Content of Fe and Pd in the bimetallic-EPS species

Catalyst	Fe (%)	Pd (%)
8.4 % Pd-Fe-EPS	7.4	8.4
2.2 % Pd-Fe-EPS	14	2.2

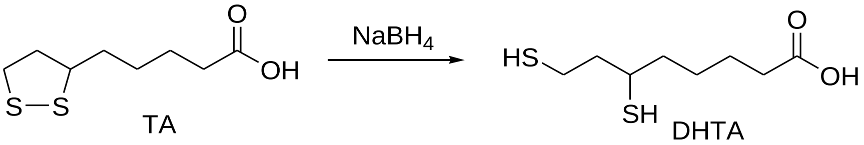
5.14.2.5 Preparation of 1.2 % Rh-EPS catalysts

Klebsiella oxytoca BAS-10 was retrieved from cryovials kept at −80 ◦C in 25% glycerol in Nutrient broth (Difco). An aliquot of 1 ml of overnight culture of BAS-10 was transferred in a media containing per liter: 2.5 g $NaHCO_3$, 1.5 g NH_4Cl, 1.5 g $MgSO_4{\bullet}7H_2O$, 0.6 g NaH_2PO_4, 0.1 g KCl, and

50 mM (14.7 g/L) Na-citrate, hereafter referred to as NAC medium, was buffered at pH 7.6 with NaOH. The culture was inoculated (1:100, v:v) and incubated at 30 ◦C under anaerobic conditions. At the beginning of the stationary growth phase (2 days, optical density > 1 at 600 nm) in static mode the BAS-10 culture was amended with 101.7 mg of $RhCl_3$ (final concentration of Rh = 50 ppm) and shaken with magnetic bar for 72 h. After this time, the desired 1.2 % Rh-EPS was recovered as reported at paragraph 5.14.2.1.

5.14.3 Preparation of water soluble specie [Rh(DHTA)Na]

5.14.3.1 Syntesis of dihydro thioctic acid (DHTA) and DHTANa



The preparation of this compound was performed by adopting the procedure described in the literature with small modifications[71].

In a 25 mL round bottom flask equipped with magnetic stirrer, 0.21 g (0.0025 mol) of Na_2CO_3 dissolved in 12 mL of water and 0.510 mg of thioctic acid (**TA**) (0.0025 mol) were introduced. After complete dissolution of acid (**TA**), the solution was cooled in an ice bath and then 0.19 g (0.005 mol) of $NaBH_4$ were slowly added. The mixture was stirred for 2 h at 4 °C. Subsequently, the mixture was acidified at pH 1 by a 2 M solution of HCl and extracted with $CHCl_3$ (3x10 mL). The recovered organic phase was dried on Na_2SO_4, filtered and the solvent was removed by rotavapor. The desired product **DHTA** was obtained in 98 % yield as a transparent liquid. DHTA was stocked at -20°C under nitrogen.

^{1}H-NMR ($CDCl_3$) δ (ppm): 10.1 (bs, 1H, OH), 2.89 (m, 1H, S-CH), 2.7 (m, 2H, S-CH_2), 2.4 (t, 2H, CH_2-COOH, j 0 7.1 Hz), 1.92-1.94 (m, 8H, $(CH_2)_4$), 1.36 (t, 1H, SH, J = 7.9), 1.31 (d, 1H, SH, J = 7.6)
^{13}H-NMR ($CDCl_3$) δ (ppm): 180.4 (COOH), 43.1 (SH-CH_2-**CH_2**-CH), 39.7 (**CH_2**-COOH), 39.1 (**CH_2**-CH-SH), 34.3 (CH-SH), 26.9 (CH-CH_2-**CH_2**), 24.7 (**CH_2**-CH_2-COOH), 22.7 (CH_2-SH)

The corresponding sodium salt was obtained by treating 0.507 g (0.0025 mol) of DHTA with 1 equivalent of Na_2CO_3 dissolved in 10 mL of distilled water.

^{1}H-NMR (H_2O/D_2O): δ (ppm): 2.92 (m, 1H, S-CH), 2.6 (m, 2H, S-CH_2), 2.08 (t, 2H, CH_2-COONa, J = 7.2 Hz), 1.85-1.78 (M, 2H, HS-CH_2-C**H_2**-CH-SH), 1.72-1.3 (m, 10H, (CH2)$_4$; 2 SH)

5.14.3.2 Synthesis of [Rh(DHTANa)]

In a 25 mL round bottom flask, equipped with magnetic stirrer, 0.010 g (0.05 mmol) of **TA** was dissolved in 10 mL of deaerated distilled water containing 1 equivalent of Na_2CO_3 under nitrogen. The mixture was then stirred until the complete salification of acid **TA**. Subsequently 0.0124 g (0.025 mmol) of $[Rh(COD)Cl]_2$ was added and the mixture was stirred until complete dissolution. The obtained red-orange Rh solution ([Rh] = 0.005 M) seems to be stable on the air but it was stocked under nitrogen at 4°C for safety.

The complex was also isolated in a solid form: the water was removed under vacuum, and the obtained solid was redissolved in methanol, filtered and reprecipitated with small additions of diethyl ether. The obtained precipitate was filtered and finally dried. The obtained orange solid is stable on the air and it was characterized by NMR:

^{1}H-NMR (H_2O/D_2O): δ (ppm): 3.61 (m, 1H, S-CH), 3.12 (m, 2H, S-CH_2), 2.08 (t, 2H, CH_2COONa, J = 7.2 Hz), 1.85-1.78 (m, 2H, HS-CH_2-C**H_2**-CH-SH), 1.72-1.3 (m, 10H, $(CH_2)_4$; 2 SH)

5.14.4 Preparation of uncommercial carboxyreductase enzyme (CAR)

5.14.4.1 Preparation of starter culture of genetically modified Escherichia Coli BL 21 (DE3)

In a 50 mL centrifuge tube, 20 mL of sterile LB broth, 20 µL of ampicillin (from 500mM stock solution) and 20 µL of chloramphenicol (from 500 mM stock solution) were introduced under sterile conditions. Subsequently, genetically modified *Escherichia Coli* BL 21 (DE3) was inoculated and it was grown up at 37°C, 250 rpm for 17 h. After 17 hours, the optical density (OD) was checked at 600 nm to determine the inoculum volume for the successive step of preparation (expression culture). For that reason, 0,5 mL of pre-culture was taken under sterile condition and diluted with 0,5 mL of HEPES buffer.For culture, it is necessary the value of 0.1 starting OD. The calculation to determine the inoculum volume to be taken from starter culture is:

$$C1 \times V1 = C2 \times V2$$

Where:

C1: OD of starter culture after 17 h.
V1: sample volume from starter culture analyzed at 600 nm.
C2: 0.1 (starting OD of expression culture).
V2: desired volume for expression culture.

The OD of starting culture after 17 h was 0.878 (for 0.5 mL to 1 mL of solution). After calculation, the necessary volume to inoculate the expression culture is 5.70 mL.

5.14.4.2 Expression culture and recovering of enzyme

In a 1 L Erlenmayer flask, 100 mL of Overnight Instant Medium TB, 100 µL of ampicillin (from 500 mM stock solution), 100 µL of chloramphenicol (from 500 mM stock solution) and 5.70 mL of starting culture was introduced under sterile condition. The culture was grown at 37°C, 250 rpm for 24 h. After 24 h, 100 µL of culture was taken and diluted with HEPES up to 1 mL. This sample was checked at 600 nm to determine the OD value and the determined OD was 8.25. This value is necessary to calculate the correct lysis volume to recover the enzyme from cell.

The expression culture was split into ten 10 mL test tube, than centrifuged at 3900 rpm for 20 minutes at room temperature. The supernatant is discarded and the precipitate (cell pellets) was recovered. The necessary volume to lysis the cell was calculated in this way:

$$\frac{(32.2 \bullet \mathrm{OD} \bullet 10)}{1000} = lysis\ volume$$

32.2: is an arbitrary value express in µL.
OD: Optical density of expression culture after 24 h.
10: volume from the cell pellets was precipitated.
1000: conversion factor.

The necessary lysis volume was 2.66 mL.

This volume was prepared by using 2.59 mL of HEPES 50 mM buffered at pH = 7.5; 53 µL of n-Hexane (corresponding at 2 % of total volume) and 13.3 µL of 200 mM solution of PMSF (the concentration into lysis volume must be 1 mM).

The pellets content in a 10 mL test tube was diluted with 2.66 mL of solution above describe and after was lysed for 20 minutes (15 s of impulse and 45 of relax time) at 0°C (ice bath) by using Ultrasound equipment.

The obtained mixture was centrifuged at 10000 rpm, 4°C for 30 minutes. The supernatant (from here denominated as CFE: cell free extract) containing the soluble CAR enzyme was recovered and stored at -20°C. The presence of CAR enzyme was determined by electrophoretic analysis and activity test. The cell pellets were also analyzed and discarded.

5.14.4.3 Purification of CAR enzyme by Ni-chromatography

To purify the CAR enzyme contained in CFE, it needs to preliminarily prepare four buffers with the following characteristics:

Buffer I: 50 mM HEPES, 300 mM of NaCl, 20 mM imidazole, final pH = 7.5.
Buffer II: 50 mM HEPES, 300 mM NaCl, 50 mM imidazole, final pH = 7.5.
Buffer III: 50 mM HEPES, 300 mM NaCl, 200 mM imidazole, final pH = 7.5.
Buffer IV: 50 mM HEPES, 300 mM NaCl, 500 mM imidazole, final pH = 7.5.

In a centrifuge tube, 2 mL of 20 % Ni-Agarose solution from stock solution in ethanol was introduced and centrifuged at 13000 rpm for 5 minutes at room temperature to remove the ethanol as supernatant. Subsequently, 1.5 mL of buffer I was introduced and the obtained mixture was centrifuged at 13000 rpm for 5 minutes at room temperature and the supernatant was discarded. These operations were replaced three times and the Ni-agarose solution for purification column was obtained. At the same time, 100 µL of 1000 mM imidazole solution was introduced into 5 mL of CFE, recovered by adopting the procedure described at paragraph 5.14.4.2 (the final concentration of imidazole was 20 mM). Then, this solution was united at the Ni-Agarose solution and this new mixture was generously mixed by vortex and was introduced into a chromatographic column for Ni-affinity chromatography. This column was washed with 5 mL of buffered II, 5 ml of buffered III and 5 mL of buffered III. The all single fractions obtained from washing with different buffer were collected and after electrophoresis analysis, the fraction obtained by washing with buffer III was introduced into dialysis membranes (cut off: 10000) then, it was introduced into a 100 mL of 50 mM Hepes solution at pH 7.5 at 4°C and maintained under these conditions for 24 h. After this time, the dialysis membrane was pierced and the contained solution was recovered into a test tube with filter and centrifuged at 3900 rpm for 20 minutes at room temperature. After this treatment, the desired CAR enzyme was recovered into the filter. Then, the enzyme was solubilized with 5.8 mL HEPES buffer at pH 7.5 and recovered by using syringe, filtered through a 0.2 μm filter and recovered into a vial. At the end, 1.45 mL of 50 % glycerol stock solution was added and the obtained solution was stored at -80°C (the final concentration of glycerol was 10 %). The purity of obtained enzyme was checked by electrophoresis analysis.

5.14.5 Preparation of salified form of Aquivion®

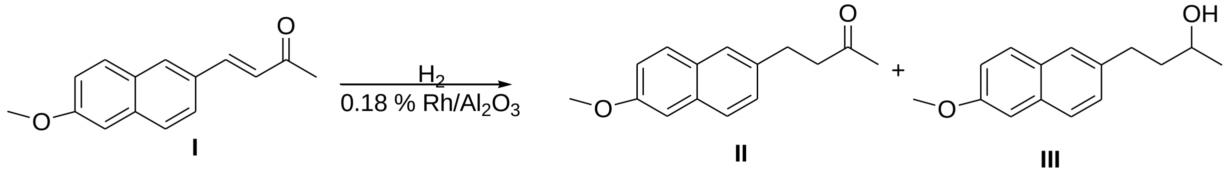
For example, the preparation of Aquivion®-Fe is here reported.

In 250 mL three necks round bottom flask equipped with mechanical stirrer and reflux condenser, 10 g (870g/eq. of $–SO_3H$) of Aquivion®, 0.214 g (0.0038 mol) of Iron and 50 mL of acetonitrile were introduced. The solution was heated at reflux for 48 h. After this time, acetonitrile was removed by rotavapor and subsequently the Aquivion®-Fe was dried under vacuum at 6 Pa. The recovered catalyst was grinded on agate mortar and stored into a bottle. The final determined Iron amount was 2.05 % w/w (theoretic: 2.14 % w/w)

Adopting the same procedure, Aquivion®-Ga and Aquivion®-In were prepared, by using 0.267 g of Ga and 0.440 g of In for 10 g of Aquivion® respectively.

5.15 Reactions

5.15.1 Hydrogenation of 4-(2-methoxynaftalen-6-yl)but-3-en-2-one (I)



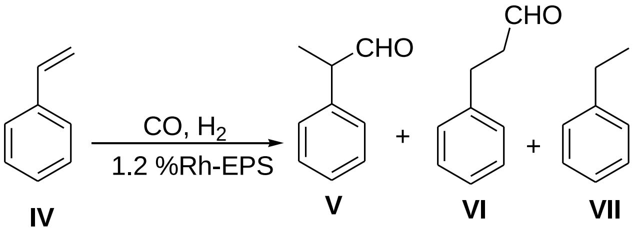
For example, here it is reported experiment 5 of Table 3.2.1, paragraph 3.2 of results and discussions.

In a Schlenk tube, 3.56 g (0.016 mol) of **I**, 0.180 g of 0.18 % Rh/Al_2O_3 (substrate **I**/catalyst molar ratio 5000/1) and 20 mL of toluene were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 0.5 MPa H_2 and stirred for 1 h at 50°C. The reactor was then cooled to room temperature and the residual gases released. The catalyst was filtered off by using a synthered glass filter and the organic phase was analyzed by GC and GC-MS. 93 % conversion of substrate **I** into product **II** was detected. The recovered catalyst was washed with diethylether (3x10 mL), dried under vacuum and recycled by adopting the above described procedure.

GC-MS m/z I: 226 $[M]^+$; 225 $[M; -H]^+$; 211 $[M, -CH_3]^+$; 183 $[M; -CH_3CO]^+$;
GC-MS m/z II: 228 $[M]^+$; 171 $[M; -C_3H_5O]^+$; 141 $[M; -C_3H_5O-CH_3O]^+$; 115 $[141; -C_2H_2]^+$
GC-MS m/z III: 230 $[M]^+$; 212 $[M; -H_2O]^+$; 197 $[M; -H_2O-CH_3]^+$; 172 $[M; -C_3H_6O]^+$; 171 $[M; -C_3H_5O]^+$; 141 $[M; -C_3H_5O-CH_3O]^+$; 115 $[141; -C_2H_2]^+$

5.15.2 Hydroformylation of Styrene (IV)

5.15.2.1 Hydroformylation of Styrene (IV) catalyzed by 1.2 % Rh-EPS



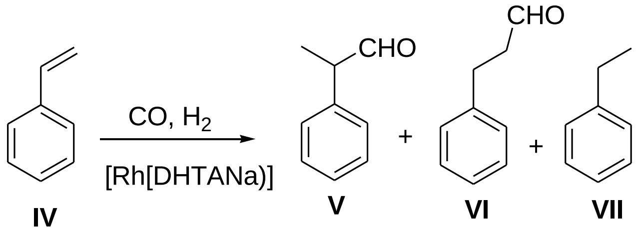
For example, here it is reported experiment 1 of Table 3.3.1, paragraph 3.3 of results and discussion.

In a Schlenk tube, 0.003 g of 1.2 % Pd-EPS (**IV**/catalyst molar ratio 700/1) were stirred under nitrogen in 2 mL of distilled water for about 10 minutes. A solution of 0.025 g (0.24 mmol) of **IV** in 2 mL of toluene was the added to the aqueous phase. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 6 MPa of syngas (CO/H_2=1) and heated at 60°C for 20 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS: 91 % conversion of **IV** was detected (85 % **V**, 6 % **VI**). The catalytic aqueous phase was recycled for further experiments adopting the above described procedure.

GC-MS m/z V: 134 $[M]^+$; 105 $[M, -HCO]^+$

GC-MS m/z VI: 134 $[M]^+$; 105 $[M, -HCO]^+$; 91 $[M, -CH_2CHO]^+$

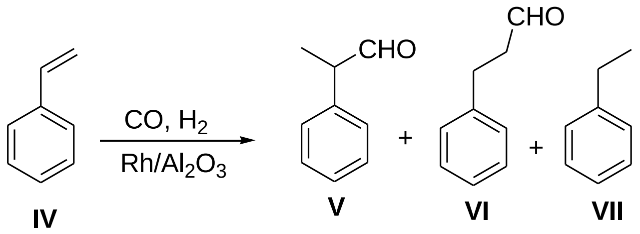
5.15.2.2 Hydroformylation of Styrene (IV) catalyzed by [Rh(DHTA)Na]



For example, here it is reported experiment 1 of Table 3.3.2, paragraph 3.3 of results and discussions.

In a Schlenk tube, 1 mL of 0.005 M solution of [Rh(DHTANa)] in 2 mL of degased distilled water, 0.52 g (0.005 mol) of styrene (**IV**) and 2 mL of toluene were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 8 MPa of syngas (CO/H_2=1) and stirred for 18 h at 80°C. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. 99 % conversion of **IV** was detected (83 % **V**, 16 % **VII**). The catalytic aqueous phase was recycled by adopting the above described procedure.

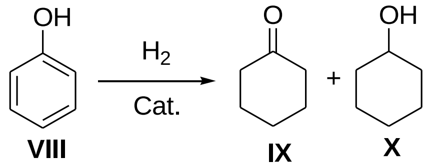
5.15.2.3 Hydroformylation of Styrene (IV) catalyzed by 0.18 % Rh/Al_2O_3



For example, here it is reported experiment 1, Table 3.3.3, paragraph 3.3 of results and discussions.

In a Schlenk tube, 0.8 g of 0.18 % of Rh/Al_2O_3 (equal to **I**/catalyst molar ratio 800/1) and 5 mL of toluene were charged. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 4 MPa of syngas (CO/H_2=1) and heated at 50°C for 20 hours under stirring. After this time, the catalyst was filtered by using a synthered glass filter, washed with diethyl ether (3x5 mL) and dried under vacuum and recycled by adopting the above describe procedure. Organic phase was analyzed by GC and GC-MS. The quantitative conversion of styrene (**IV**) was detected, (93 % **V**, 6 % **VI**).

5.15.3 Hydrogenation of phenol (VIII) catalyzed 0,18 % Rh/Al_2O_3 or 0.27 % Pd/Al_2O_3



For example, here it is reported experiment 1 of Table 3.4.2, paragraph 3.4 of results and discussions.

In a Schlenk tube, 0.010 g of 0.18 % Rh/Al_2O_3 (molar ratio **VIII**/cat 1000/1), 0.016 g (0.17 mmol) of **VIII** and 10 mL of distilled water were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 0.5 MPa H_2 and stirred for 1 h at 60°C. The reactor was then cooled at room temperature and the residual gas was released. The mixture was treated with 10 mL of diethyl ether and the organic phase was dried over Na_2SO_4 and analyzed by GC and GC-MS. Conversion was 88 % (76 % **IX**, 12 % **X**).

Under the same conditions the reaction was carried out by using 0,010 g of 0.28 % Pd/Al_2O_3 (**VIII**/catalyst molar ratio 1000/1), 0.025 g of **VIII**, 10 mL of distilled water and after 1 h 26 % conversion into **IX** was detected. Furthermore, a recycling test was also carried out (run 1, Table 3.4.2) at 50°C for 20 h, by adopting the procedure above described. In this case, the water phase containing the 0.18 % Rh/Al_2O_3 catalyst was recycled twice.

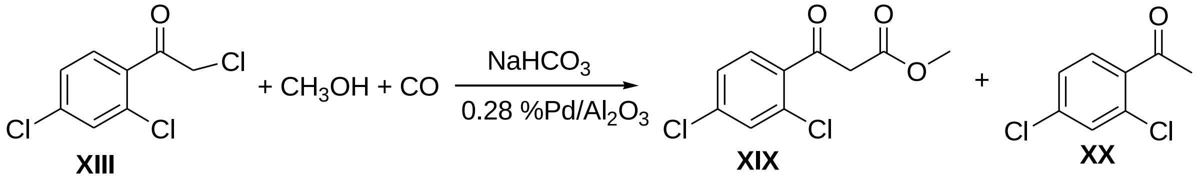
GC-MS m/z VIII: 94 $[M]^+$; 77 $[M; -OH]^+$

GC-MS m/z IX: 100 $[M]^+$; 57 $[M; -C_3H_7]^+$

GC-MS m/z (X): 98 $[M]^+$; 69 $[M; -C_2H_5]^+$; 55 $[M; -C_3H_7]^+$

5.15.4 Synthesis of β-ketoesters by carboxymethylation of chloro-ketones

5.15.4.1 Carboxymethylation of 2-chloro-1-(2,4-dichlorophenyl)ethanone (XIII) catalyzed by homemade 0.28 % Pd/Al_2O_3



For example, here it is reported experiment 1 of Table 3.5.5 of paragraph 3.5 of results and discussions.

In a Schlenk tube, 0.152 g of 0.28 % Pd/Al_2O_3 (molar ratio **XIII**/cat 500/1) and 10 mL of MEK were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 1 MPa CO and stirred for 2 h at 30°C. The reactor was then cooled to room temperature, the residual gases released and, under nitrogen, 0.444 g (0,002 mol) of **XIII**, 0.252 g of $NaHCO_3$ (base/**XIII** molar ratio 1.5/1) and 0.512 g of methanol (d = 0.79 g/mL, methanol/**XIII** molar ratio 8/1) were introduced. The autoclave was then pressurized with 2.5 MPa of CO and heated to 100°C under stirring for 4 hours. After this time, the reactor was cooled at room temperature and the residual gas released. The crude mixture was diluted with 5 mL of diethyl ether, washed with 5 mL of distilled water and centrifuged. This operation was repeated twice. The recovered catalyst was washed with 5 mL of diethyl ether and centrifuged three times, than dried under vacuum. The recovered organic phase was separate from the aqueous phase by centrifugation, dried on Na_2SO_4 and analyzed by GC and GC-M. 87 % conversion into **XIV** was found. The desired product was purified by silica column by using a mixture 1:1 Ethyl Acetate/n-Hexane. The pure desired product **XIV** was obtained in 70 % yield and characterized with ^1H-NMR. The recovered catalyst was recycled by adopting the above reported procedure.

GC-MS m/z XIV: 246 $[M]^+$; 186 $[M; -C_2H_3O_2]^+$; 173 $[M; -C_3H_6O_2]^+$; 145 $[M; -C_4H_5O_2]^+$; 109 $[145; -HCl]^+$, 74 $[109; -Cl]^+$.

1H-NMR ($CDCl_3$), δ (ppm) XIV: 7.81 (d, 1H, CH arom); 7.46 (s, 1H, CH arom); 7.30 (d, 1H, CH arom), 3.68 (s, 3H; CH_3); 3.49 (s, 2H, CH_2)

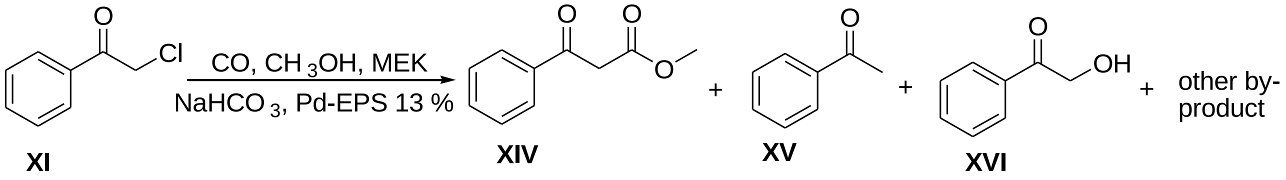
The carboxymethylation of 2-chloro-1-phenylethanone (**XI**) and 2-chloro-1-(4-chlorophenyl)ethanone (**XII**) was also carried out by adopting the procedure above reported. For example (run 3, Table 3.5.2) by carbonylation of **XI**, 37 % conversion in **XIV**, was detected after 5 h and by carbonylation of 2-chloro-1-(4-chlorophenyl)ethanone (**XII**), 72 % conversion was obtained (67 % **XII**, 5 % by-products).

GC-MS m/z XIV: 178 $[M]^+$; 105 $[M; -C_3H_5O_2]^+$; 77 $[M; C_4H_5O_3]^+$; 51 $[77; -C_2H_2]^+$

GC-MS m/z XII: 212 $[M]^+$; 139 $[M; -C_3H_5O_2]^+$; 111 $[M; -C_4H_5O_3]^+$; 75 $[111, -HCl]^+$

^{1}H-NMR ($CDCl_3$), δ (ppm) XIV: 7.84 (d, 1H, CH arom); 7.46 (d, 1H, CH arom); 3.93 (s, 3H, CH_3), 3.68 (s, 2H; CH_2)

5.15.4.2 Carboxymethylation of 2-chloro-1-phenylethanone (XI) catalyzed by 13 % (Pd-EPS)$_{anaerb}$



For example, here it is described the experiment 1 reported in Table 3.5.3, paragraph 3.5 of results and discussions.

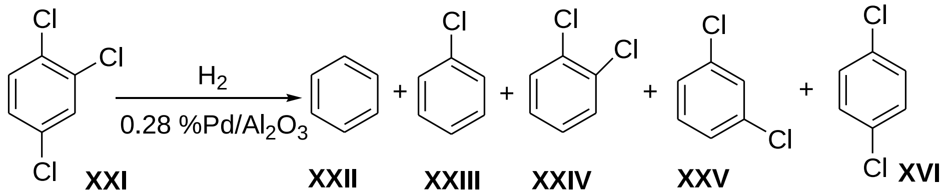
In a Schlenk tube, 0.003 g of 13% (Pd-EPS)$_{anaerob}$ (molar ratio **XI**/cat 500/1) and 2 mL of distilled water were added and stirred for 10 minutes. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 1 MPa CO and stirred for 2 h at 30°C. The reactor was then cooled to room temperature, the residual gases released and, under nitrogen, 0.3 g (0.002 mol) of **XI**, 0.245 g of $NaHCO_3$ (base/**XI** molar ratio 1.5/1) and 0.49 g of methanol (d = 0,79 g/mL, methanol/**XI** molar ratio 8/1) and 7 mL of MEK were introduced under nitrogen. The autoclave was then pressurized with 4.5 MPa of CO and heated to 100°C under stirring for 20 hours. After this time, the reactor was than cooled at room temperature and the residual gas released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. 99 % conversion was detected (51 % **XIV**, 5 % **XV**, 19 % **XVI**, 24 % other by-product).

GC/MS m/z XVI: 136 $[M]^+$; 105 $[M; -CH_2OH]^+$; 77 $[M; - COCH_2OH]^+$; 51 $[77; -C_2H_2]^+$

GC-MS m/z XV: 120 $[M]^+$; 105 $[M; -CH_3]^+$; 77 $[M; -CH_3CO]^+$; 51 $[77; -C_2H_2]^+$

5.15.5 Hydrodechlorination of 1,2,4 trichlorobenzene (XXI)

5.15.5.1 Hydrodechlorination of 1,2,4 trichlorobenzene (XXI) catalyzed by 0.28 % Pd/Al_2O_3



For example, here it is described the experiment 1 reported in Table 3.6.2, paragraph 3.6 of results and discussions.

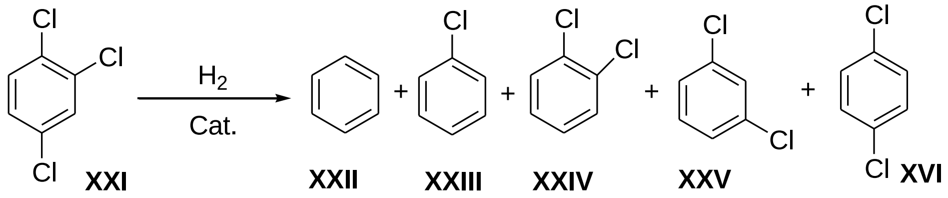
In a Schlenk tube, 0.050 g of 0.28 % Pd/Al_2O_3 (molar ratio **XXI**/cat 100/1), 0.024 g (0.13 mmol) of **XXI**, 0.033 g of $NaHCO_3$ (base/**XXI** molar ratio 3/1) and 7 mL of distilled water were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 0.2 MPa of H_2 and stirred for 20 h at 30°C. The reactor was then cooled at room temperature and the residual gas was released. The mixture was extracted with 5 mL of diethyl ether three times and the recovered organic phase was dried over Na_2SO_4 and analyzed by GC and GC-MS. Quantitative conversion into benzene (**XXII**) was detected.

All the reactions reported at Table 3.6.1 and 3.6.2 at paragraph 3.6 were carried out by adopting the above described procedure.

GC-MS m/z XXII:77 $[M]^+$

Adopting the same procedure, the reaction was also performed in distilled water, or in xylene or in neat condition by using 5 % Pd/C Chimet as catalyst and PMHS as H-donor. The conditions and the obtained results are reported in Table 3.6.3, paragraph 3.6 of results and discussions.

5.15.5.2. Hydrodechlorination of 1,2,4 trichlorobenzene (XXI) catalyzed by different Pd-based catalysts (1.7 % (Pd-EPS)$_{aerob}$, 13 % (Pd-EPS)$_{anaerob}$, 8.4 % Pd-Fe-EPS and 2.2 % Pd-Fe-EPS)



For example, here is reported the hydrodechlorination of **XXI** catalyzed by 13 % Pd-EPS, Table 3.6.4 of paragraph 3.6 of results and discussions.

In a Schlenk tube, 0.026 g of 13% (Pd-EPS)$_{anaerob}$ (molar ratio **XXI**/cat 8/1) and 2 mL of distilled water were stirred for ten minutes. Subsequently, 0.356 g (0.0196 mol) of **XXI**, and 1 mL of THF were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 3 MPa of H_2 and stirred for 20 h at 60°C. The reactor was then cooled at room temperature and the residual gas was released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS analyses. The catalytic aqueous phase was recycled for further experiments adopting the above described procedure. 76 % of conversion was detected (9 % **XXII**, 19 % **XXIII**, 36 % **XXIV**; 6 % **XXV**; 14% % **XVI**).

GC-MS m/z XXII: 112 $[M]^+$; 76 $[M; -Cl]^+$

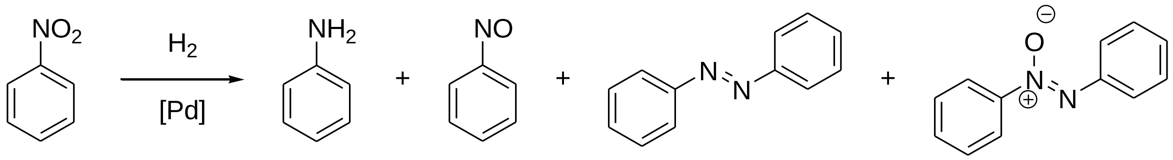
GC-MS m/z XXIII, XXIV, XXV, XVI: 147 $[M]^+$; 112 $[M; -Cl]^+$; 76 $[M; -2Cl]^+$ (The different kind of isomers were identified by pure standard injections)

GC-MS m/z XXI: 181 $[M]^+$; 146 $[M; -Cl]^+$; 111 $[M; -2Cl]^+$; 76 $[M;- 3Cl]^+$

Same procedure was adopted for the reaction carried out by using 1.7 % (Pd-EPS)$_{aerob}$, 8.4 % Pd-Fe-EPS and 2.2 % Pd-Fe-EPS, respectively.

5.15.6 Reduction of nitro aromatic compounds

5.15.6.1 Hydrogenation of nitrobenzene (XXVII) catalyzed by biogenerated Pd-EPS catalysts



XXVII **XXVIII** **XXIX** **XXX** **XXXI**

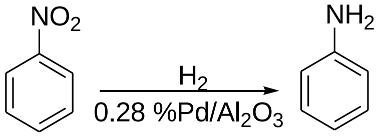
For example, here it is described the experiment 1 reported in Table 3.7.1, paragraph 3.7 of results and discussions.

In a Schlenk tube, 0.006 g of 1.7 % $(Pd\text{-}EPS)_{aerob}$ (**XXVII**/catalyst molar ratio 1000/1) was stirred under nitrogen in 2 mL of distilled water for about 10 minutes. A solution of 0.120 g (0.00098 mol) of **XXVII** in 2 mL of THF was then added to the aqueous phase. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 5 MPa of H_2 and heated at 80°C for 20 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. 98 % Conversion of substrate into aniline (**XXVIII**) was detected. The catalytic aqueous phase was recycled for further experiments adopting the above described procedure.

GC-MS m/z XXVIII: 93 $[M]^+$; 76 $[M; -NH_3]^+$; 66 $[M; -HCN]^+$

Adopting the same procedure, the reaction was carried out under different conditions (Table 3.7.1) also by using 8.4 % Pd-Fe-EPS.

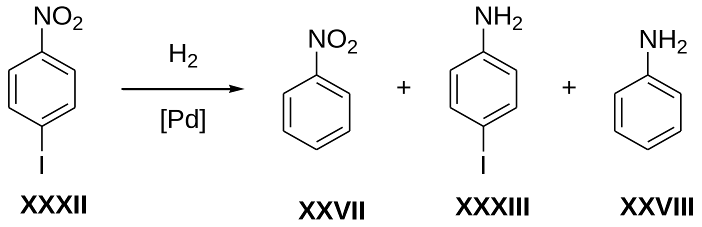
5.15.6.2 Hydrogenation of nitrobenzene (XXVII) catalyzed by 0.28 % Pd/Al_2O_3



For example, here it is described the experiment 6 reported in Table 3.7.2, paragraph 3.7 of results and discussions.

In a Schlenk tube, 0.065 g (0.0053 mol) of **XXVII**, 0.2 g of 0.28 % Pd/Al_2O_3 (**XXVII**/catalyst molar ratio 100/1) and 10 mL THF were introduced. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 0.2 MPa of H_2 and stirred for 1 h at 25°C. After this time, the residual gas was released, and the catalyst was filtered on a synthered glass filter, washed with 10 mL of diethylether and dried under vacuum. The recovered organic phase was analyzed by GC and GC–MS. Quantitative conversion of substrate **XXVII** into **XXVIII** was detected. The recovered catalyst was recycled by adopting the above described procedure.

5.15.6.3 Hydrogenation of 1-iodo-4-nitrobenzene (XXXII) catalyzed by biogenerated Pd-EPS catalysts



For example, here it is described the experiment 1 reported in Table 3.7.3, paragraph 3.7 of results and discussions.

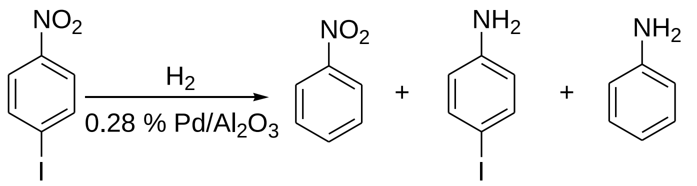
In a Schlenk tube, 0.006 g of 1.7 % $(\text{Pd-EPS})_{\text{aerob}}$ (**XXXII**/catalyst molar ratio 1000/1) were stirred under nitrogen in 2 mL of distilled water for about 10 minutes. A solution of 0.244 g (0.98 mmol) of **XXXII** in 2 mL of THF was added to the aqueous phase. The Schlenk tube was the transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 3 MPa of H_2 and heated at 80°C for 24 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated, dried on

Na_2SO_4 and analyzed by GC and GC–MS. 69 % Conversion of substrate into **XXVII** was detected. The catalytic aqueous phase was recycled for further experiments adopting the above described procedure.

GC-MS m/z XXVII: 123 $[M]^+$; 93 $[M; -NO]^+$; 77 $[M; -NO_2]^+$; 65 $[M; -CONO]^+$; 51 $[M; -C_2H_4NO_2]^+$

Adopting the same procedure, the reaction was also performed by using 1.7 % activated $(Pd\text{-}EPS)_{aerob}$ or 8.4 % Pd-Fe-EPS as catalyst. It was also studied the influence of different types of base and the use of a pH = 10 buffer. All reaction conditions are reported at Table 3.7.4.

5.15.6.4 Hydrogenation of 1-iodo-4-nitrobenzene (XXXII) catalyzed by 0.28 % Pd/Al_2O_3

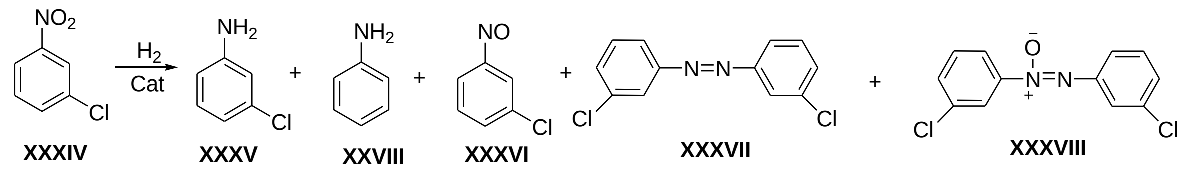


XXXII **XXVII** **XXXIII** **XXVIII**

For example, here it is described the experiment 1 reported in Table 3.7.7

In a Schlenk tube, 0.488 g (0.00196 mol) of **XXXII**, 0.148 g of 0.28 % Pd/Al_2O_3 (**XXXII**/catalyst molar ratio 500/1) and 10 mL of distilled water were introduced. The Schlenk tube was then transferred into a 150 ml stainless steel autoclave under nitrogen, pressurized with 3 MPa of H_2 and stirred for 24 h at 80°C. The reactor then was cooled at room temperature and the residual gas was released. The water solution was extracted three times with 10 mL of diethyl ether and the collected organic phase was dried on Na_2SO_4 and analyzed by GC and GC-MS. Quantitative conversion into nitrobenzene (**XXVII**) was detected. The water phase was recycled by adopting the above described procedure.

5.15.6.5 Hydrogenation of 1-chloro-3-nitrobenzene (XXXIV) catalyzed by 0.28 % Pd/Al_2O_3 and 0.27 % Pt/Al_2O_3



For example, here it is described the experiment 9 reported in table Table 3.7.8, paragraph 3.7 of results and discussions.

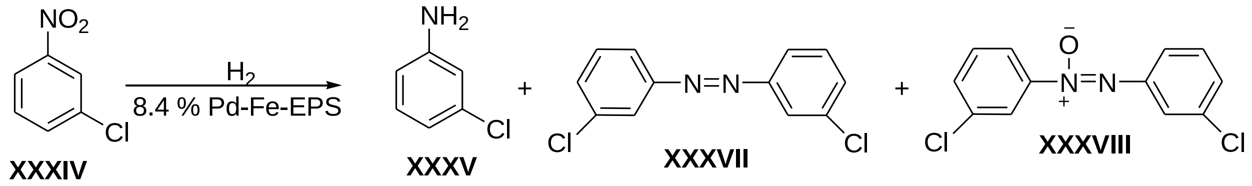
In a Schlenk tube, 0.385 g (0.0024 mol) of **XXXIV**, 0.200 g of 0.28 % Pd/Al_2O_3 (**XXXIV**/catalyst molar ratio 500/1) and 10 mL of THF were introduced. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 0.5 MPa of H_2 and stirred for 1 h at 50°C. The reactor then was cooled at room temperature and the residual gas was released. The catalyst was filtered on a synthered glass filter and washed three times with 10 mL of diethyl ether and dried under vacuum. The collected organic phase was dried on Na_2SO_4 and analyzed by GC and GC-MS. 90 % Conversion of substrate **XXXIV** was detected (84 % 1-chloro-3-nitroaniline (**XXXV**) and 6 % of aniline (**XXXV**)). The recovered catalyst was recycled by adopting the above described procedure.

GC-MS m/z XXXV: 127 $[M]^+$; 100 $[M; -HCN]^+$; 92 $[M; -Cl]^+$; 65 $[M; -Cl-HCN]^+$

The same procedure was adopted by using 0.27 % Pt/Al_2O_3. The reaction was carried out at 50°C, 0.5 MPa of hydrogen for 7 hours, by using a substrate **XXXIV**/molar ratio 100/1.

Adopting to the same procedure, the reaction was also performed in the presence of $NaHCO_3$. The reaction conditions are reported in Table 3.7.9 paragraph 3.7.

5.15.6.6 Hydrogenation of 1-chloro-3-nitrobenzene catalyzed by 8.4 % Pd-Fe-EPS



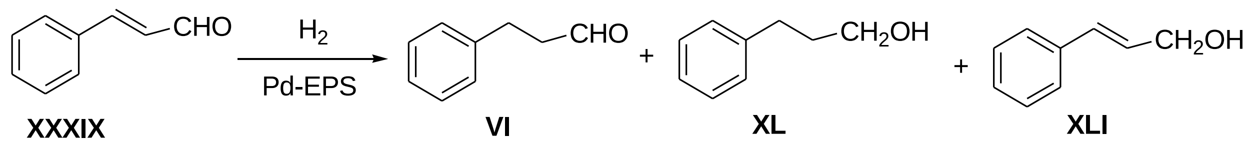
For example, here it is described the experiment 1 reported in Table 3.7.10, paragraph 3.7 of results and discussions.

In a Schlenk tube, 0.003 g of 8.4 % Pd-Fe-EPS (**XXXIV**/catalyst molar ratio 500/1) was stirred in 3 mL of distilled water for 10 minutes. Subsequently, 0.189 g (0.0012 mol) of **XXXIV** dissolved in 3 mL of THF were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 3 MPa of H_2 and stirred for 24 h at 80°C. The reactor then was cooled at room temperature and the residual gas was released.
Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. 81 % Conversion of **XXXIV** was detected (47 % **XXXV**, 9 % **XXXVII** and 25 % **XXXVIII**) The catalytic aqueous phase was recycled for further experiments adopting the above described procedure.

GC-MS m/z XXVII: 250 $[M]^+$; 111 $[M; -C_6H_4ClN_2]^+$; 75 $[M; -C_6H_5Cl_2N_2]^+$
GC-MS m/z XXVIII: 267 $[M]^+$; 231 $[M; -Cl]^+$; 111 $[M; -ClC_6H_4N_2O]^+$

5.15.7 Hydrogenation of trans-cinnamaldehyde (XXXIX)

5.15.7.1 Hydrogenation of trans-cinnamaldehyde (XXXIX) catalyzed by activated 1.7 % (Pd-EPS)$_{aerob}$



For example, here it is described the experiment 1 reported in table Table 3.8.1; paragraph 3.8 of results and discussions.

In a Schlenk tube, 0.012 g of activated 1.7 % (Pd-EPS)$_{aerob}$ (**XXXIX**/catalyst molar ratio 1000/1) was stirred in 4 mL of distilled water for 10 minutes. Subsequently, 0.260 g (0.00196 mol) of **XXXIX**, dissolved in 4 mL of THF, was added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 2 MPa of H_2 and stirred for 6 h at 50°C. The reactor was then cooled at room temperature and the residual gas was released.

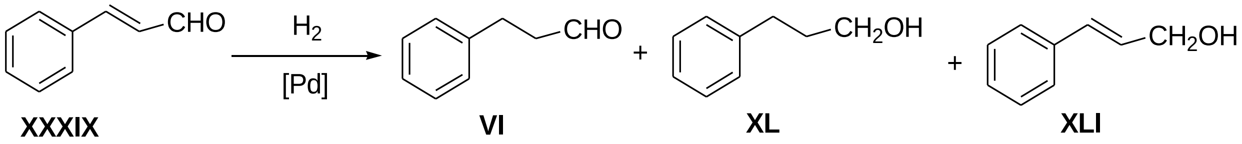
Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. 96 % Conversion of **XXXIX** was detected (89 % of **VI**, 6 % **XL**, 1 % **XLI**) The catalytic aqueous phase was recycled for further experiments adopting the above described procedure.

GC-MS m/z VI: 134 $[M]^+$; 105 $[M; -HCO]^+$; 91 $[M; -CH_2HCO]^+$; 78 $[M; -HCH_2CH_2HCO]^+$

GC-MS m/z XL: 136 $[M]^+$; 118 $[M; -H_2O]^+$; 105$[M, -CH_2OH]^+$; 91 $[M; -CH_2CH_2OH]^+$; 77 $[M; –CH_2CH_2CH_2OH]^+$

GC-MS m/z XLI: 134 $[M]^+$; 118 $[M; -H_2O]^+$; 105$[M; -CH_2OH]^+$; 91 $[M; -CH_2CH_2OH]^+$

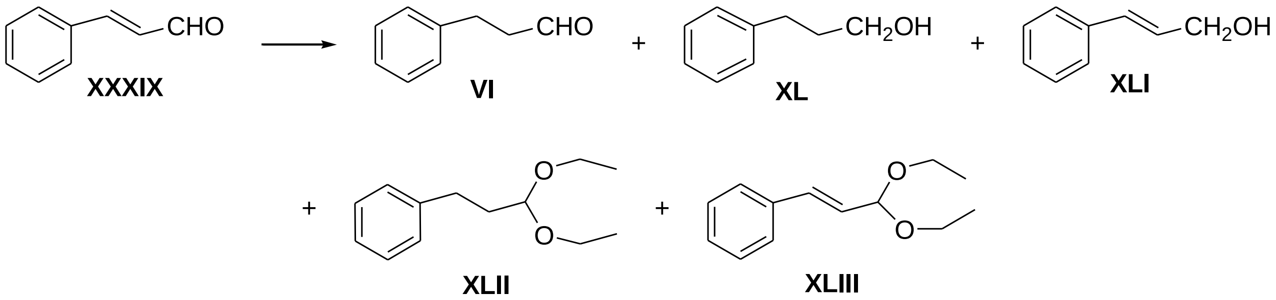
5.15.7.2 Hydrogenation of trans-cinnamaldehyde (XXXIX) catalyzed by homemade 0.28 % Pd/Al$_2$O$_3$, 0.5 % Pd/Al$_2$O$_3$, 5 % Pd/C Chimet, and 0.5 % and 0.6 % Pd-BASF and 4.3 % Pd-ENCATS



For example, here it is described the experiment 1 reported in table Table 3.8.2, paragraph 3.8 of results and discussions.

In a Schlenk tube, 0.010 g of homemade 0.28 % Pd/Al$_2$O$_3$ (**XXXIX**/catalyst molar ratio 2500/1), 0.09 g (0.00068 mol) of **XXXIX** and 8 mL of toluene were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 0.2 MPa of H$_2$ and stirred for 20 h at 35°C. The reactor then was cooled at room temperature and the residual gas was released. The mixture was analyzed by GC and GC-MS and 99 % conversion into 3-phenylpropanal (**VI**) was found.
Adopting the same procedure, the reaction was also performed by using 0.5 % Pd/Al$_2$O$_3$ 5 % Pd/C Chimet, and 0.5 % and 0.6 % Pd-BASF and 4.3 % Pd-ENCATS. The reaction conditions and the obtained results are reported at Table 3.8.4 and 3.8.6.

5.15.7.3 Hydrogenation of trans-cinnamaldehyde (XXXIX) catalyzed by 0.28 % Pd/Al$_2$O$_3$, 0.5 % Pd-BASF, 0.6 % Pd-BASF and 0.5 % Pd/Al$_2$O$_3$ by using an autoclave endowed with mechanical stirrer



For example, here it is described the experiment 4 reported in Table 3.8.5
In a 300 mL stainless steel reaction vessel equipped with mechanical stirrer, under a nitrogen purge, 5.95 g (0.045 mol) of **XXXIX**, 0.055 g of 0.28 % Pd/Al$_2$O$_3$ (molar ratio **XXXIX**/cat 32000/1)

and 94 mL of ethanol were charged. The autoclave then was pressurized at 2 MPa of H_2 and heated at 60 °C for 2 h. The reactor was then cooled at room temperature and the residual gas was released. The mixture was analyzed by GC and GC-MS. The conversion was 92 % (14 % **VI**; 8 % **XL**, 67 % **XLII** and 3 % **XLIII**).

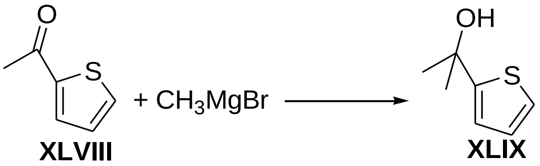
Adopting the same procedure, the reaction was also performed by using 0.5 % Pd-BASF, 0.6 % Pd-BASF and 0.5 % Pd/Al_2O_3.

GC-MS m/z XLII: 208 $[M]^+$; 162 $[M; -C_2H_6O]^+$; 117 $[162; -C_2H_6O]^+$; 103 $[117; -CH_2]^+$; 91 $[C_7H_7]^+$

GC-MS m/z XLIII: 206 $[M]^+$; 161 $[M; -C_2H_6O]^+$; 133 $[161; -C_2H_4]^+$; 105 $[133; -CO]^+$;76 $[C_6H_5]^+$

5.15.8 Synthesis of Lioral™ (LXI$_a$) (and similar structure compound, Helional™ (LXIX) and their precursor

5.15.8.1 Synthesis of 2-(thiophen-2-yl)propan-2-ol (XLIX)



In a 1000 mL round bottom flask equipped with mechanical stirrer, 50 g (0.40 mol) of **XLVIII** and 200 mL of dry diethyl ether were placed under nitrogen. Subsequently, 160 mL of 3M solution of CH_3MgBr in Et_2O was added dropwise in 30 minutes at 5 °C. After this time the reaction was warmed at 25°C until complete conversion of 2-acetylthiophene (**XLVIII**). The reaction was monitored using silica-TLC (eluent: n-hexane/ Et_2O 8/2) and GC. At the end of the reaction the mixture was treated with a saturated solution of NH_4Cl (50 mL) and the aqueous phase was extracted twice with diethyl ether (3x30 mL). The collected organic phase was dried with Na_2SO_4, filtered and concentrated by rotavapor to give 54 g (95 % yield) of alcohol **XLIX** as a yellow liquid.

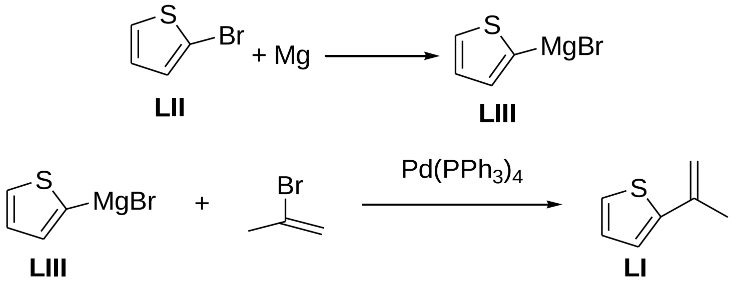
GC-MS m/z XLIX: 142 $[M]^+$; 83 $[142, -C(CH_3)_2OH]^+$

^{1}H-NMR ($CDCl_3$, 300 MHZ), δ (ppm): 1.7 (s, 6H, 2 CH_3), 2.0 (s, 1H, OH), 6.9 (m, 1H, CH arom), 7.2 (m, 2H, CH arom)

5.15.8.2 Synthesis of 2-(prop-1-en-2-yl)thiophene (LI)

5.15.8.2.1 Synthesis of 2-(prop-1-en-2-yl)thiophene (**LI**) by cross-coupling reaction

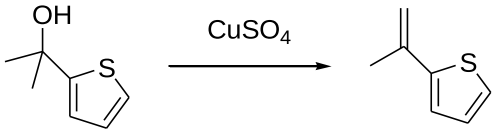
The synthesis was carried out as reported in literature[151].



In a 100 mL two neck round bottom flask, equipped with magnetic stirrer and reflux condenser, 0.298 g (0.012 mol) of magnesium, 10 ml of anhydrous diethyl ether and a small amount of iodine were added. Subsequently 2 g (0,012 mol) of **LII** dissolved in 10 mL of anhydrous diethyl ether were added by a dropping funnel, under nitrogen. At the end of the addition, the mixture was warmed at reflux and the reaction was performed until magnesium disappeared.

In a 100 mL two neck round bottom flask equipped with magnetic stirrer and reflux condenser, 0.140 g (0.123 mmol, **LIII**/catalyst molar ratio 100/1) of $Pd(PPh_3)_4$, 1.09 mL (0.012 mol) of 2-bromo prop-1-ene (d = 1.362 g/mL) and 20 mL of anhydrous diethyl ether were added under nitrogen and the Grignard intermediate prepared in the previous step, were added by dropping funnel. When the addition was finished, the mixture was warmed at reflux. During the reaction, a formation of solid product that precipitated on the bottom of the flask was observed.

5.15.8.2.2 Synthesis of 2-(prop-1-en-2-yl)thiophene (**LI**) by dehydration with $CuSO_4$



XLIX **LI**

The synthesis was carried out as reported in literature for similar compounds[152]. Anhydrous copper (II) sulfate was prepared by heating $CuSO_4 \cdot 5\ H_2O$ at 280°C for 2 days. The off-white solid was obtained and it was stored in an oven at 150°C.

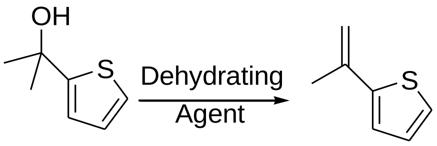
In a 25 mL round bottom flask equipped with magnetic stirrer, Vigreux column and condenser, 2 g (0,014 mol) of **XLIX** and 1.67 g (0.0105 mol) of $CuSO_4$ were introduced. The mixture was heated at 60 °C under 0.1 mmHg of vacuum until the distillation of the desired product **LI** into a bottom flask receiver stopped. After 30 minutes, 0.66 g of **LI** (38 % yield) were recovered.

GC-MS (LI) m/z: 124$[M]^+$; 109 $[124\text{-}CH_3]^+$; 83 $[124\text{-} C(CH_2CH_3)]^+$

^{1}H-NMR ($CDCl_3$, 300 MHZ), δ (ppm): 7.05 (m, 1H, CH arom); 7.3(m, 2H, CH arom); 5.45 (s, 1H, CH_2); 5.0 (s, 1H, CH_2); 2.2 (s, 3H, CH_3)

Adopting the same procedure, the reaction was also performed under different conditions reported in Table 3.9.2, paragraph 3.9 of results and discussions.

5.15.8.2.3 Synthesis of 2-(prop-1-en-2-yl)thiophene (**LI**) by dehydration with different dehydrating agents



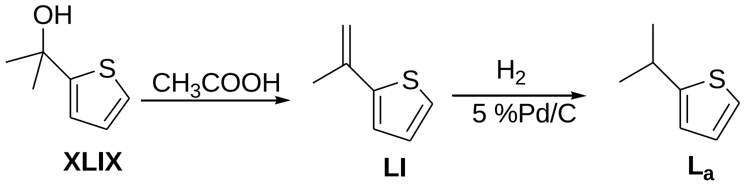
XLIX **LI**

For example, here is reported the procedure adopted for experiment 2 reported in Table 3.9.3.

In a 100 mL round bottom flask equipped with magnetic stirrer, 5 g (0.035 mol) of 2-(thiophen-2-yl)propan-2-ol (**XLIX**), 1.5 g of acid coal superwj and 30 mL of n-hexane were introduced. The reaction was carried out at 25°C for 24 h. The mixture was analyzed by GC and GC-MS and quantitative conversion of **XLIX** was detected (55 % **LI**, 44 % undesired by-product).

Adopting the same procedure, the reaction was also performed by using Aquivion® as dehydrating agent (run 1, Table 3.9.3, paragraph 3.9 of results and discussions).

5.15.8.2.4 Synthesis of 2-(prop-1-en-2-yl)thiophene (**LI**) by dehydration with CH_3COOH/KI system and reduction of the olefin intermediate (**LI**) catalyzed by 5 % Pd/C Chimet

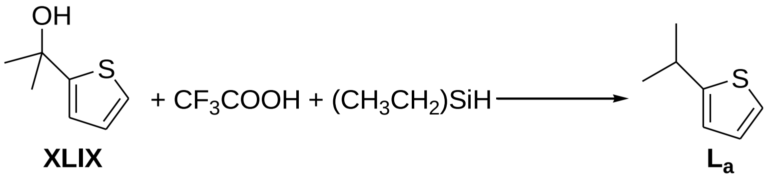


In a round jacket bottom flask equipped with magnetic stirrer and reflux condenser, 5 g (0.035 mol) of 2-(thiophen-2-yl)propan-2-ol (**XLIX**), 0.6 g (0.004 mol) of KI, 7 g of water solution of Na_2SO_3 (50 % w/w), 40 mL of CH_3COOH and 40 mL of n-Hexane were added. The mixture was heated at 50°C for 2 h, and monitored by GC and GC-MS. 50 % Conversion into the desired olefin **LI** was detected. After reaction, acetic acid was separated from n-Hexane by a separatory funnel and the organic phase was washed with water until the pH of the recovered fraction was neutral.

The organic phase was transferred into a Schlenk tube and, by considering 50 % conversion into **LI**, 0.516 g of 5 % Pd/C Chimet (50 % wet) were added. The Schlenk tube was transferred into a 150 mL stainless steel autoclave under nitrogen and pressurized with 5 MPa of H_2. The reaction was carried out at 25°C for 24 hours under stirring. The conversion, monitored by GC and GC-MS, into 2-isopropylthiophene (**L$_a$**) was 80%. The crude mixture was filtered to remove the catalyst and it was washed 3 times with 10 mL of diethyl ether. Subsequently, the solvent was removed by distillation at atmospheric pressure and **L$_a$** was recovered by distillation at 6 Pa and 33°C. The pure desired product was obtained in 40 % yield.

5.15.8.3 Synthesis of 2-isopropylthiophene (L_a)

5.15.8.3.1 Synthesis of 2-isopropylthiophene (**L_a**) by hydrogenation/dehydration with Triethylsilane and Trifluoroacetic acid



The synthesis was carried out as reported in the literature[150].

In a 50 mL two neck round bottom flash equipped with magnetic stirrer, 2 g (0,014 mol) of **XLIX**, and 2.24 g (0,021 mol) of triethylsilane (d = 0,728 g/mL) were dissolved in 10 mL of anhydrous dichloromethane at 0°C. Subsequently, 2.39 g (0,021 mol) of trifluoroacetic acid (d = 1,535 g/mL) dissolved in 10 mL of anhydrous dichloromethane were added dropwise. When the addition was complete the reaction mixture was stirred 1 h at room temperature. The reaction was monitored by GC and GC-MS and after 1 h the quantitative conversion of **XLIX** was determined. It was found 40 % formation of **L_a** and 59 % of an undesired by-product.

The crude mixture was washed with 20 mL of water and the organic phase was separated by a separatory funnel. The recovered organic phase was dried over Na_2SO_4, and after filtration, dichloromethane was removed by atmospheric distillation and the desired product was recovered by under vacuum distillation at 6 Pa and 33 °C. The isolated yield of the desired product was 13 %.

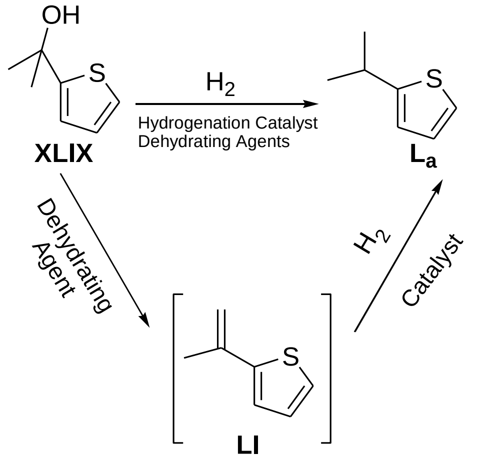
GC/MS (L_a) m/z: 126 $[M]^+$; 111 $[M, -CH_3]^+$; 83 $[M, -CH(CH_3)_2]^+$

^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30 (m ,2H, CH arom); 7.05 (m, 1H, CH arom); 3.2 (m, 1H, CH); 1.40 (d, 6H, $2CH_3$)

The reaction was also performed by using PMHS as H-donor. The obtained results are reported at paragraph 3.9.2.2

5.15.8.3.2 Synthesis of 2-isopropylthiophene (**L$_a$**) by a combined reaction of dehydration and hydrogenation

For example here the adopted procedure to synthetize 2-isopropylthiophene (**L$_a$**) by using Amberlist-15®H$^+$ form and 5 % Pd/C as catalyst is reported.



In a Schlenk tube, 5 g (0.035 mol) of **XLIX**, 1.032 g of 5 % Pd/C Chimet (50 % wet; substrate **XLIX**/catalyst molar ratio 140/1), 0.5 g of Amberlist-15® H$^+$ form, 0.05 g of BHT, 1 mL of isopropylether and 30 mL of isooctane were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 0.5 MPa H_2 and stirred for 24 h at 25°C. After this time the residual gas was released and the mixture was analysed by GC and GC-MS. 99 % Conversion of the starting material **XLIX** was detected (80 % desired product **L$_a$**, 19 % undesired by-product). The mixture was filtered to remove Amberlist-15® H$^+$ and 5 % Pd/C Chimet and both were washed with 10 mL of diethyl ether three times. The recovered organic phase was washed with 6 M KOH solution and subsequently with water until the pH was neutral, than dried on Na_2SO_4. Subsequently, Na_2SO_4 was filtered off and the solvent was removed by distillation at atmospheric pressure. The desired product (**L$_a$**) was obtained by distillation under vacuum at 6 Pa and 33°C. **L$_a$** was obtained in 50 % yield.

Adopting the same procedure, the reaction was also carried out by using different dehydrating agent such as acetic acid, Montmorillonite K-10, Acid coal super wj, $CuSO_4$ and the obtained results and reaction conditions are reported in Table 3.9.4. In the same table, one experiment (run 5) was carried out by using PMHS as H-donor. The procedure was also adopted when the reaction was performed by using 1 % Pd/acid Al_2O_3 and 1 % Pd/acid coal as catalytic systems (Table 3.9.5)

5.15.8.3.3 Synthesis of 2-isopropylthiophene (**L$_a$**) by a combined reaction of dehydration and hydrogenation catalyzed by acetic acid and Pd(OAc)$_2$

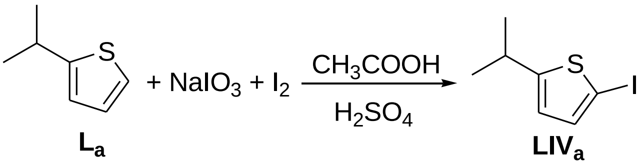
For example, here the procedure used for run 2 reported in Table 3.9.6, is described.

In a Schlenk tube, 0.032 g of Pd(OAc)$_2$ and 10 mL of THF were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 1 MPa of H_2 and stirred for 1 h at room temperature. After this time the residual gas was released and 2 g (0.014 mol) of **XLIX** (**XLIX**/catalyst molar ratio 100/1), 1 mL of CH_3COOH (d = 1.05 g/mL) and 10 mL of n-Hexane were introduced in the Schlenk tube, then it was transferred into a 150 mL stainless steel autoclave under nitrogen. The reaction was performed at 0.5 MPa and 50°C for 24 under stirring. After this time, the autoclave was cooled at room temperature, the residual gas was released and the crude mixture was analyzed by GC and GC-MS. 80 % Conversion of the starting material **XLIX** was detected (40 % **L$_a$**, 40 % undesired by-product).

Adopting the same procedure, the reaction was also performed by using acetic anhydride as dehydrating agent and both conditions and obtained results are reported in Table 3.9.6.

5.15.8.4 Halogenation of 2-isopropylthiophene (L$_a$), 2-ethylthiophene (L$_b$) and benzo[d][1,3]dioxole (LXX)

5.15.8.4.1 Ioduration of 2-isopropylthiophene (**L$_a$**), 2-ethylthiophene (**L$_b$**) and benzo[d][1,3]dioxole (**LXX**)



For example, here the iodination of 2-isopropylthiophene (**L$_a$**) is reported.

In a 250 mL jacket round bottom flask with two necks, equipped with magnetic stirrer and reflux condenser, 10 mL of water, 2.66 g of $NaIO_3$ (0.014 mol), 4.82 g of I_2 (0,019 mol), 20 mL of glacial acetic acid, 5 g (0.040 mol) of **L$_a$** and 30 mL of ethyl acetate and 1.4 mL of sulfuric acid at 98 % were introduced. The reaction was carried out for three hours at 30°C, and monitored by GC and GC-MS. The quantitative conversion of **L$_a$** was detected, corresponding to 98 % into the desired product **LIV$_a$** and 1 % of a by-product. After three hours, the reaction mixture was treated with 30 mL of deionized water and 30 mL of 50 % sodium hydroxide solution until neutral pH.

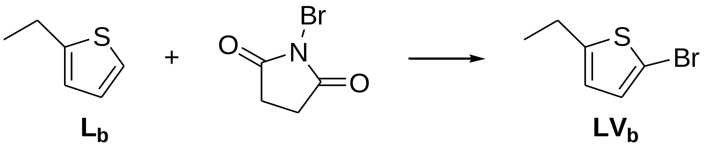
Subsequently, 10.63 g (0.043 mol) of sodium thiosulfate were added and the organic phase was separated. The aqueous phase was extracted five times with 30 mL of ethyl acetate. The organic phases were recovered and dried on Na_2SO_4. After filtration, the solvent was removed by rotavapor and the desired product was obtained by distillation at reduced pressure (94°C, 13 Pa mmHg). **LIV$_a$** was obtained in 80 % yield.

GC-MS m/z LIV$_a$: 252 $[M]^+$; 237 $[M, -CH_3]^+$; 126 $[M, -I]^+$; 110 $[M, -I, -CH_3]^+$
^{1}H-NMR ($CDCl_3$), δ (ppm): 7.06 (d, 1H, CH arom); 6.51 (d, 1H, CH arom); 3.17 (m, 1H, CH); 1.32 (d, 6H, $2CH_3$)

Adopting the same procedure, the iodination of 2-ethylthiophene (**L$_b$**) and benzo[d][1,3]dioxole (**LXX**) were performed. After 3 h, 92 % conversion of **L$_b$** into **LIV$_b$** were detected by GC and GC-MS and after work-up, pure **LIV$_b$** was obtained in 81 % yield. After 20 h, quantitative conversion of **LXX** into **LXXIII** were detected by GC and GC-MS and after work, pure desired compound **LXXIII** was obtained in 88 % yield.

GC-MS m/z LIV$_b$: 238 $[M]^+$; 112 $[M; -I]^+$
^{1}H-NMR ($CDCl_3$), δ (ppm) LIV$_b$: 6.90 d (1H, CH arom); 6.56 (d, 1H, CH arom); 2.59 (q, 2H, CH_2), 1.24 (t, 3H, CH_3)
GC-MS m/z LXXIII: 248 $[M]^+$; 223 $[M; -CH_3]^+$; 121 $[M; -I]^+$
^{1}H-NMR ($CDCl_3$), δ (ppm) LXXIII: 6.97 (d, 1H, CH arom); 6.94 (s, 1H, CH arom); 6.70 (d, 1H, CH arom), 5.80 (s, 2H; CH_2).

5.15.8.4.2 Bromination of 2-ethylthiophene (**L$_b$**) by using N-Bromosuccinimide



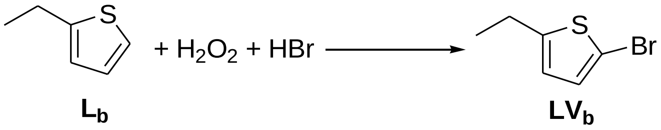
In a 50 mL round bottom flask equipped with magnetic stirrer, 1 g (0,009 mol) of 2-ethylthiophene (**L$_b$**), 1.60 g (0.009 mol) of NBS were dissolved in 10 mL of chloroform and 10 mL of acetic acid. The reaction was carried out for 2 hours at room temperature and monitored by GC and GC-MS. The detected conversion **L_b** was 99 % (97 % in 2-bromo-5-ethylthiophene (**LV_b**) 2 % in an other monobrominate isomer). The crude mixture was treated with 30 mL of water and neutralized with 30 % solution of NaOH. Subsequently, the aqueous phase was extracted three times with chloroform (3 x 5 mL) and the recovered organic phases were dried over Na_2SO_4. After filtration and concentration by rotavapor, the desired product was obtained by distillation at reduced pressure (6 Pa, 30°C). The desired product was obtained in 81 % yield.

GC-MS m/z LV_b: 190 $[M]^+$, 175 $[M, -CH_3]^+$; 110 $[M, -Br]^+$; 95 $[M, -Br\ e\ CH_3]^+$.

^{1}H-NMR ($CDCl_3$), δ (ppm): 6.86 (d, 1H, CH arom); 6.57 (d, 1H, CH arom); 2.50 (q, 2H, CH_2); 1.30 (t, 3H, CH_3).

5.15.8.4.3 Bromination of 2-ethylthiophene (**L_b**) and benzo[d][1,3]dioxole (**LXX**) by HBr/H_2O_2 system

For example, here the bromination of 2-ethylthiophene (**L_b**) was reported
The reaction was performed as reported in the literature[155].

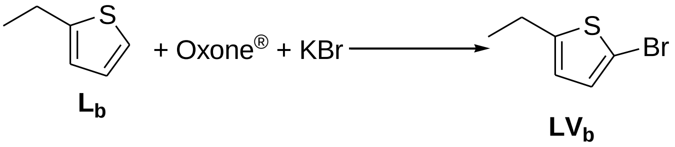


In a 50 mL round bottom flask equipped with magnetic stirrer and covered with aluminum foil, 1 g (0,009 mol) of 2-ethylthiophene (**L_b**) was suspended in 3 mL of CH_3CN and 7 mL of water. A 48 % aqueous solution of HBr (0.005 mol, 0.56 mL) and 30 % aqueous solution of H_2O_2 (0.005 mol, 0.5 mL) was gradually added in 90 minutes. The reaction was carried out for 4 hours at room temperature and monitored by GC and GC-MS. The detected conversion of 2-ethylthiophene **L_b** was 91 % (87 % in 2-bromo-5-ethylthiophene (**LV_b**) and 4 % in di-brominated by-product). The crude mixture was treated with 10 mL of diethyl ether and the aqueous phase was separated. Subsequently, the aqueous phase was extracted three times with diethyl ether (3 x 5 mL) and the recovered organic phases were dried over Na_2SO_4. After filtration and concentration by rotavapor, the desired product was obtained by distillation at reduced pressure (6 Pa, 30°C). The desired product (**LV_b**) was obtained in 81 % yield.

Adopting the same procedure, the reaction was also performed on benzo[d][1.3]dioxole (**LXX**). After 4 h, 90 % conversion of substrate **LXX** into the desired **LXXIII** was detected. After purification bu distillation, the desired product was obtained in 80 % yield.

GC-MS m/z LXX: 200 $[M]^+$; 121 $[M; -Br]^+$
^{1}H-NMR ($CDCl_3$), δ (ppm) LXX: 7.14 (d, 1H, CH arom); 7.20 (s, 1H, CH arom); 6.62 (d, 1H, CH arom); 6.00 (s, 2H; CH_2).

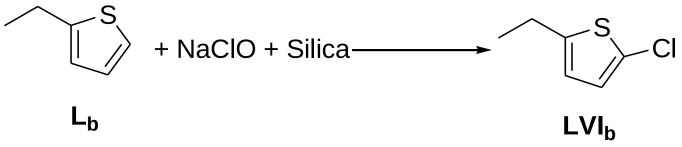
5.15.8.4.4 Bromination of 2-ethylthiophene (**L_b**) and benzo[d][1,3]dioxole (**LXX**) by Oxone® and KBr



For example, here the procedure to brominate 2-ethylthiophene (**L_b**) is reported.
The reaction was performed as reported in the literature[156].

In a 50 mL round bottom flask equipped with magnetic stirrer and a dropping funnel, 1 g (0.009 mol) of **L_b** was suspended in 3 mL of CH_3CN and 7 mL of water. Subsequently, 5.53 g (0.0086 mol) of Oxone® dissolved in 10 mL of distilled water were slowly dropped in 30 minutes. The reaction was carried out at room temperature and monitored by GC and GC-MS. After 2 h, 90 % conversion of **L_b** was detected (82 % **LV_b**, 8 % dibrominated compound). The crude mixture was quenched with aqueous sodium thiosulfate (2.71 g equal to 0.0172 mol in 10 mL) and extracted three times with 10 mL of diethylether. The combined organic phase were washed with 10 mL of distilled water and dried over Na_2SO_4. After filtration, the crude mixture was concentrated by rotavapor and distilled under vacuum at 6 Pa and 30°C. The desired product was obtained in 60 %.
Adopting the same procedure, the reaction was also performed on [1.3]benzo[d]dioxole (**LXX**). After 6 h, 92 % conversion of substrate into **LXXIII** was detected. After work-up, pure 2-bromo-[1.3]benzo[d]dioxole (**LXXIII**) was obtained in 75 % yield.

5.15.8.4.5 Chlorination of 2-ethylthiophene (**L$_b$**) by silica and sodium hypochlorite



In a 100 mL two neck round bottom flask equipped with magnetic stirrer and reflux condenser, 3 g (0.0267 mol) of 2-ethylthiophene (**L$_b$**), 25 mL of chloroform and 3.8 g of silica were added. The mixture was heated at 50°C and a solution of 5 % NaClO at pH 8-9 (buffered by $NaHCO_3$) was dropped in 1 hour. The reaction was carried out for 24 h and monitored by GC and GC-MS. The conversion into the desired product **LVI$_b$** was 90 %. The crude mixture was filtered to remove silica and subsequently the organic phase was separated from the aqueous phase. The aqueous phase was extracted with 10 mL of dichloromethane three times. The organic phase was recovered, dried on Na_2SO_4, filtered and concentrated by rotavapor. The desired product was obtained by distillation at reduced pressure at 13 Pa 50°C. The desired product was obtained **LVI$_b$** in 60 % yield.

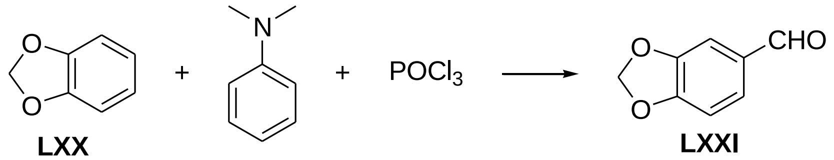
GC-MS m/z LVI$_b$: 146 $[M]^+$; 131 $[M; -CH_3]^+$; 111 $[M; -Cl]^+$

^{1}H-NMR ($CDCl_3$) δ(ppm) LVI$_b$: 6.73 (d, 1H, CH arom); 6.56 (d, 1H, CH arom); 2.76 (q, 2H, CH_2), 1.29 (t, 3H; CH_3)

GC-MS m/z LVII$_b$: 128 $[M]^+$; 113 $[M, -CH_3]^+$

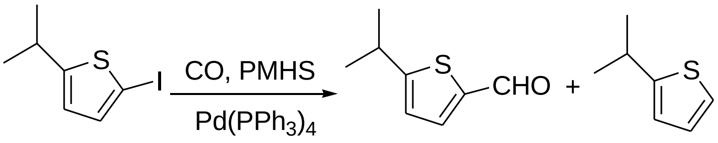
5.15.8.5 Synthesis of 5-isopropylthiophene-2-carbaldehyde ($LVIII_a$), 5-ethylthiophene-2-carbaldehyde ($LVIII_b$) and benzo[d][1,3]dioxole-5-carbaldehyde (piperonal) (LXXI)

5.15.8.5.1 Synthesis and benzo[d][1,3]dioxole-carbaldehyde (piperonal (**LXXI**)) by Vilsmeyer reaction


LXX **LXXI**

In a jacket round bottom flask, equipped with mechanical stirrer and reflux condenser, 9.45 g (0.070 mol) of MFA (d = 1.095 g/mL) were introduced under nitrogen at 0°C. Subsequently, 10.73 g (0.070 mol) of $POCl_3$ (d = 1.675 g/mL) were added by dropping funnel. The mixture was stirred for 40 minutes at 0°C in order to obtain the Vilsmeyer reactive. After this time, 8.18 g (0.067 mol) of **LXX** were added by a dropping funnel and the mixture was then heated at 90 °C for 24 h. After this, time only 32 % conversion in desired product **LXXI** was found.

5.15.8.5.2 Synthesis of 5-isopropylthiophen-2-carbaldehyde (**$LVIII_a$**) and piperonal (**LXXI**) by reductive carbonylation in the presence of PMHS, Na_2CO_3 and $Pd(PPh_3)_4$


LIV_a **$LVIII_a$** **L_a**

For example, here the procedure adopted for experiment 3 reported in Table 3.9.8, paragraph 3.9.5.1.

In a Schlenk tube, 0.051 g of $Pd(PPh_3)_4$ (**LIV_a**/catalyst molar ratio 180/1), 10 mL of Me-THF, 3.36 mL of PMHS, 1.70 g of Na_2CO_3 (base/**LIV_a** molar ratio 2/1) and 2 g (0.008 mol) of **LIV_a** were introduced under nitrogen. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 10 MPa of CO and heated at 80°C for 20 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. The crude mixture was filtered and the recovered organic phase was analyzed by GC and GC-MS. Quantitative conversion of substrate **LIV_a** into **$LVIII_a$** was detected. The pure compound **$LVIII_a$** was obtained by distillation under vacuum (6 Pa, 54 °C) in 75 % yield.

GC-MS m/z $LVIII_a$: 154 $[M]^+$; 139 $[M, -CH_3]^+$; 125 $[M, -CHO]^+$; 111 $[125, -CH_3]^+$

^{1}H-NMR ($CDCl_3$), δ(ppm) $LVIII_a$: 9.84 (s, 1H, CHO); 7.62 (d, 1H, CH arom); 6.95 (d, 1H, CH); 3.24 (m, 1H, CH); 1.37 (d, 6H, $2CH_3$)

Adopting the same procedure the reaction was also performed under the conditions reported in Table 3.9.8.

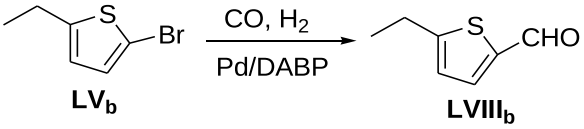
The same procedure was followed by using 5-iodobenzo[d][1,3]dioxole (**LXXIII**) as substrate. After 20 h, the conversion into the desired product **LXXI** was 66 % and after distillation under vacuum a 53 % yield was obtained.

GC-MS m/z LXXI: 150 $[M]^+$; 149 $[150 -H]^+$; 121 $[149 -CO]^+$; 91 $[121 -CH_2O]^+$

^{1}H-NMR ($CDCl_3$), δ(ppm) LXXI: 6.10 (s, 2H, CH_2); 6.95 (d, 1H, CH, J=7.9 Hz); 7.35 (d, 1H, CH, J=1.5 Hz); 7.43 (dd, 1H, CH, J_1=7.9 Hz, J_2=1.5 Hz); 9.8 (s, 1H, CHO)

5.15.8.5.3 Synthesis of 5-ethylthiophene-2-carbaldehyde (**LVIII$_b$**) and benzo[d][1,3]dioxole-carbaldehyde (piperonal) (**LXI**) by carbonylation catalyzed by Pd(OAc)$_2$/Di(1-adamantyl)-*n*-butylphosphine (DABP)

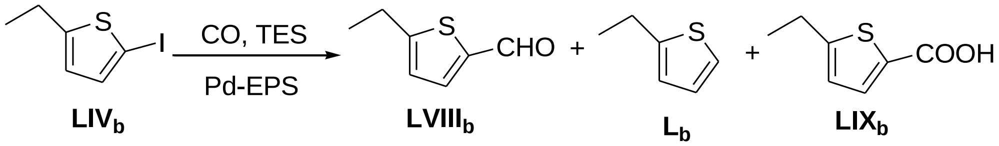
For example, here the synthesis of 5-ethylthiophene-2-carbaldehyde (**LVIII$_b$**) is reported:



In a Schlenk tube, 0.235 g of Pd(OAc)$_2$ (**LV$_b$**/catalyst molar ratio 4/1) and 0.055 g (**LV$_b$**/DABP molar ratio 1.3/1) of DABP were dissolved in 5 mL of toluene under nitrogen. After 10 minutes, 1 g (0.004 mol) of substrate **LV$_b$** and 0.375 g (substrate **LV$_b$**/base molar ratio 1.3/1) were added. The Schlenk tube was the transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 1 MPa of syngas (CO/H$_2$ = 1) and heated at 100°C for 24 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. The organic phase was analyzed by GC and GC–MS. 99 % Conversion of substrate **LV$_b$** was detected (12 % **L$_a$**, 87 % **LVIII$_b$**).

Adopting the same procedure, the reaction was also performed on 5-bromobenzo[d][1,3]dioxole (**LXXIV**) as the substrate and after 24 h, 98 % conversion into desired product **LXI** was detected.

5.15.8.5.4 Synthesis of 5-ethylthiophene-2-carbaldehyde (**LVIIIb**) by reductive formylation catalyzed by 1.7 % (Pd-EPS)aerob



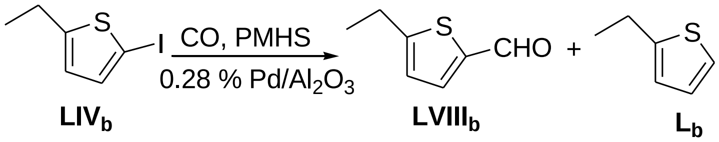
For example, here the procedure adopted for the experiment 3 reported in table 3.9.10 paragraph 3.9.5.1 is reported.

In a Schlenk tube, 0.005 g of 1.7 % (Pd-EPS)$_{aerob}$ (**LIV$_b$**/catalyst molar ratio 1000/1) were stirred under nitrogen in 2 mL of distilled water for about 10 minutes. A solution of 0.025 g (0.0001 mmol) of **LIV$_b$** in 2 mL of Me-THF, 0.0212 g (0.0002 mol) of Na$_2$CO$_3$, an 0.11 mL of TES (d = 0.728 g/mL) were added. The Schlenk tube was the transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 5 MPa CO and heated at 80°C for 24 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. Conversion of **L$_b$** was quantitative (88 % **LVIII$_b$**, 3 % **L$_b$**, 8 % **LIX$_b$**). The aqueous phase was recycled for further experiments by adopting the above described procedure.

GC-MS m/z L$_b$: 112 $[M]^+$; 97 $[M; -CH_3]^+$
GC-MS m/z LVIII$_b$: 140 $[M]^+$; 125 $[M; -CH_3]^+$; 111 $[M, -HCO]^+$; 97 $[M, -CH_3CH_2\ HCO]^+$.
GC-MS m/z LIX$_b$: 156 $[M]^+$; 141 $[M, -CH_3]^+$; 139 $[M, -OH]^+$; 111 $[M, -COOH]^+$; 97 $[M, -CH_3CH_2\ COOH]^+$

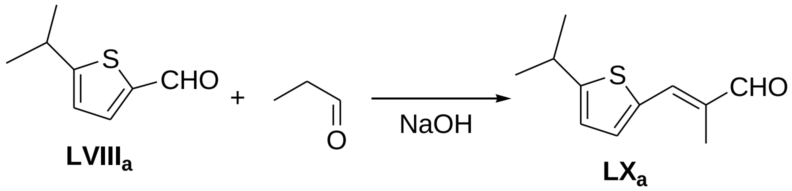
Adopting the same procedure, the reaction was also performed under the conditions reported in Table 3.9.10, paragraph 3.9.5.1.

5.15.8.5.5. Synthesis of 5-ethylthiophene-2-carbaldehyde (**LVIII$_b$**) by reductive formylation catalyzed by homemade 0.28 % Pd/Al_2O_3



In a Schlenk tube, 0.100 g of homemade 0.28 % Pd/Al_2O_3 (**LIV$_b$**/catalyst molar ratio 50/1), a solution of 0.031 g (0.00013 mmol) of **LIV$_b$** in 10 mL of Me-THF, 0.028 g (0.00026 mol) of Na_2CO_3, an 0.054 mL of PMHS were added. The Schlenk tube was the transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 5 MPa of CO and heated at 80°C for 24 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. The organic phase was analyzed by GC and GC–MS. 68 % conversion of substrate **LIV$_b$** into **LVIII$_b$** was detected.

5.15.8.6 Synthesis of 3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (LX_a) and 3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (LXXII)



In a 100 mL two neck jacket bottom flask equipped by magnetic stirrer and reflux condenser, 2 g (0.013 moli) of **LVIII$_a$** 25 mL of methanol and 0.25 g of sodium hydroxide were introduced at room temperature under nitrogen. The mixture was warmed at 60°C and 0.83 g (0.014 mol) of propionaldehyde dissolved in 15 mL of methanol were added dropwise in 40 minutes. Subsequently, the reaction was carried out for one hour at 60°C. The conversion of substrate **LVIII$_a$** was monitored by GC and GC-MS and 85 % conversion into **LX$_a$** was detected. The mixture was then cooled at 0°C and neutralized with 37 % hydrochloric acid. Methanol was removed by rotavapor and the residue was dissolved in diethyl ether and washed twice with 20 mL of distilled water. The recovered organic phase was dried on Na_2SO_4, filtered and concentrated by rotavapor. The pure desired compound **LX$_a$** was obtained by distillation under vacuum at 6 Pa and 62°C in 62 % yield.

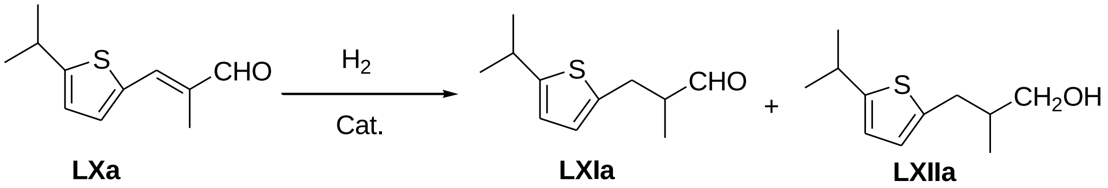
GC-MS (m/z) LX$_a$: 194 $[M]^+$; 179 $[M, -CH_3]^+$; 151 $[M, -(CH_3)_2CH]^+$
^{1}H-NMR ($CDCl_3$), δ (ppm) LX$_a$: 9.52 (s, 1H, CHO); 7.24 (s, 1H, CH=C); 7.22 (d, 1h, CH arom.); 6.90 (d, 1H, CH arom.); 3.22 (m, 1H, isopropyl CH); 2.18 (s, 3H, CH_3); 1.40 (d, 6H, $2CH_3$)

Adopting the same procedure, the reaction was also performed on piperonal (**LXXI**) as substrate: after 3 h, 71 % conversion into the desired compound was detected and after purification silica column (n-Hexane/Ethyl Acetate 8/2) pure aldehyde **LXXII** was obtained in 58 % yield.

GC-MS (m/z) LXXII: 190 $[M]^+$; 161 $[190, -CHO]^+$; 131 $[161, -CH_2O]^+$; 103 $[131, -C_2H_4]^+$; 77 $[190, -C_5H_7O_3]$; 51 $[190, -C_7H_7O_3]$
^{1}H-NMR ($CDCl_3$, 300 MHZ), δ (ppm) LXXII: 2.07 (s, 3H, CH_3); 6.04 (s, 2H, CH_2); 6.90 (d, 1H, CH, J=7.8 Hz); 7.07 (d, 1H, CH, J1=7.8 Hz); 7.09 (s, 1H, CH); 7.16 (s, 1H, CH); 9,50 (s, 1H, CH)

5.15.8.7 Hydrogenation of 3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (LX$_a$) and 3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (LXXII)

5.15.8.7.1 Hydrogenation of 3-(5-isopropylthiophen-2-yl)-2-methylacrylaldehyde (***LX$_a$***) and 3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (***LXXII***) catalyzed by Pd-EPS species



For example, here the procedure adopted for the experiment 3 reported in Table 3.9.11, paragraph 3.9.7 of results and discussions is reported.

In a Schlenk tube, 0.0024 g of 1.7 % activated (Pd-EPS)$_{aerob}$ (**LX$_a$**/catalyst molar ratio 500/1) were stirred under nitrogen in 4 mL of distilled water for about 10 minutes. Subsequently, a solution of 0.100 g (0.00196 mmol) of **LX$_a$** in 4 mL of THF were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 4 MPa of H_2 and heated at 50°C for 22 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. Conversion of **LX$_a$** was 73 % (72 % **LXI$_a$**, 1 % **LXII$_a$**). The aqueous phase was recycled for further experiments adopting the above described procedure.

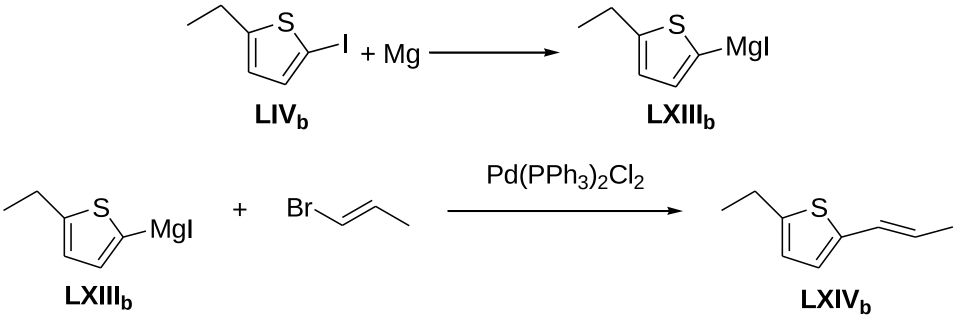
GC-MS m/z LXI$_a$: 196 $[M]^+$; 139 $[M; -CH(CHO)CH_3]^+$
GC-MS m/z LXII$_a$: 198 $[M]^+$; 126 $[M; -CH_2CH(CH_3)CH_2OH]^+$

Adopting the same procedure, the reaction was also performed on 3-(benzo[d][1,3]dioxol-6-yl)-2-methylacrylaldehyde (**LXXII**) as substrate under the conditions reported in Table 3.10. After 22 h, 83 % conversion into the desired product (**LXIX**) was observed by GC and GC-MS analysis.

GC-MS m/z LXIX: 192 $[M]^+$; 135 $[M, -CH(CH_3)CHO]^+$; 122 $[M, -CH_2CH(CH_3)CHO]^+$
GC-MS m/z LXXV: 194 $[M]^+$; 135 $[M, -CH_2CH(CH_3)CH_2OH]^+$

5.15.8.8 Synthesis of olefin 2-ethyl-5-(prop-1-enyl)thiophene (LXIV$_b$) and 2-allyl-5-ethylthiophene (LXV$_b$) by cross-coupling

The synthesis was carried out as reported in the literature[151].



In a 100 mL two neck round bottom flask equipped with magnetic stirrer and reflux condenser 0.237 g (0.010 mol) of magnesium, 20 mL of anhydrous diethyl ether and a small amount of iodine were placed under nitrogen. Subsequently 2 g (0.010 mol) of 5-ethyl-2-iodothiophene (**LIV$_b$**) dissolved in 10 mL of anhydrous ethyl ether were added by dropping funnel, always under nitrogen. At the end of the addition, the mixture was warmed at reflux and the reaction was carried out until magnesium was consumed.

Subsequently, in a 100 mL three neck round bottom flask equipped with magnetic stirrer, reflux condenser and dropping funnel, 0.068 g (0.1 mmol) of Pd(PPh$_3$)$_2$Cl$_2$ (**LIV$_b$**/catalyst molar ratio 100/1), 0.83 mL (0.01 mol) of 1-bromo prop-1-ene (d = 1.413 g/mL) and 20 ml of anhydrous diethyl ether were placed. Then, the intermediate Grignard reagent prepared in a previous step, was added by dropping funnel at room temperature. The mixture was then warmed at reflux. After two hours, 92 % conversion into the desired compound **LXIV$_b$** was detected, 2 % of **L$_b$** and 6 % of high-boiling point byproduct were also found. The crude mixture was then washed with 50 mL of a saturated ammonium chloride solution in an ice bath. Subsequently, the organic phase was separated and the aqueous phase was extracted four times with 10 mL of diethyl ether. The collected organic phases were dried on Na$_2$SO$_4$. After filtration, the solvent was removed by rotavapor and the desired product was obtained by distillation at reduced pressure (89°C, 13 Pa). The pure desired product **LXIV$_b$** was obtained in 63 % yield.

GC-MS m/z LXIV$_b$: 152 $[M]^+$; 137 $[M; -CH_3]^+$; 123 $[M; -CH_2CH_3]^+$

^{1}H-NMR ($CDCl_3$), δ (ppm): 6.82 (d, 1H, CH arom); 6.72 (d, 1H, CH arom); 6.05 (m, 1H, CH), 5.88 (m, 1H, $CH(CH_3)=CH$), 5.64 (m, 1H, $CH(CH_3)=CH$), 2.50 (q, 2H, CH_2), 1.32 (d, 3H, CH_3), 1.28 (t, 3H, CH_3)

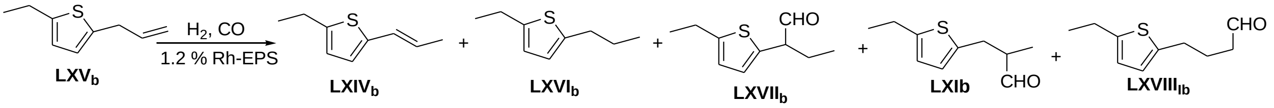
Adopting the same procedure (**LXV$_b$**) was obtained in 65 % yield.

GC-MS m/z LXV$_b$: 152 $[M]^+$; 137 $[M; -CH_3]^+$; 123 $[M; -CH_2CH_3]^+$
^{1}H-NMR ($CDCl_3$), δ (ppm) LXV$_b$: 6.9 (s, 2H, 2CH arom.), 6.0 (m, 1H, CH_2-**CH**=CH_2), 5.1 (m, 2H, **CH_2**=CH), 3.0 (d, 2H, **CH_2**-CH=CH_2), 2.50 (q, 2H, CH_2), 1,28 (t, 3H, CH_3)

5.15.8.9 Hydroformylation reaction

5.15.8.9.1. Hydroformylation of 2-ethyl-5-(prop-1-enyl)thiophene (**LXIV$_b$**) and 2-allyl-5-ethylthiophene (**LXV$_b$**) catalyzed by 1.2 % Rh-EPS

For example, here the procedure for hydroformylation of 2-allil-5-ethylthiophene (**LXV$_b$**) is reported.



For example, here the procedure adopted for the experiment 3 reported in Table 3.9.13, paragraph 3.9.9 of results and discussions.

In a Schlenk tube, 0.006 g of 1.2 % Rh-EPS (**LXV$_b$**/catalyst molar ratio 500/1) were stirred in 2 mL of distilled water for 10 minutes. Subsequently, 0.110 g (0.73 mmol) of **LXV$_b$** and 2 mL of toluene were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 8 MPa of syngas ($CO/H_2=1$) and heated at 100 °C for 20 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated, dried on Na_2SO_4 and analyzed by GC and GC–MS. 99 % Conversion of substrate **LXV$_b$** was detected (3 % **LXIV$_b$**, 3 % **LXVII$_b$**, 35 % **LXI$_b$**, 46 % **LXVIII$_b$)**. The catalytic aqueous phase was recycled for further experiments adopting the above described procedure.

GC-MS m/z LXI$_b$: 182 $[M]^+$; 125 $[M; -CH(CHO)CH_3]^+$

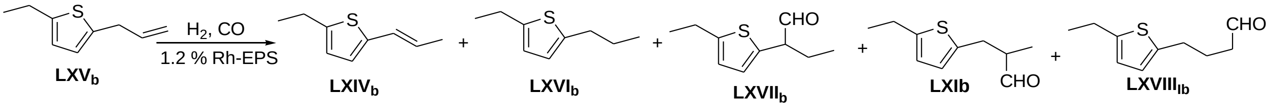
GC-MS m/z LXVIII$_b$: 182 $[M]^+$; 138 $[M; -CH_3CHO]^+$; 123 $[M, -CH_3$ and $-CH_3CHO]^+$

GC-MS m/z LXVII$_b$: 182 $[M]^+$;153 $[M; -CH_3CHO]^+$; 125 $[M; -CH(CHO)CH_3]^+$; 97 $[125; -CH_2CH_3]^+$

Adopting the same procedure, the reaction was also carried out on **LXIV$_b$** as substrate. The reaction was performed under the conditions reported in table 3.9.12. After GC and GC-MS analysis 49 % conversion of substrate (**LXIV$_b$**) was detected 6 % **LXVI$_b$;** 21 % **LXVII$_b$;** 22 % **LXI$_b$.**

GC-MS m/z LXVI$_b$: 154 $[M]^+$; 137 $[M; -CH_3]^+$; 125 $[M; -CH_2CH_3]^+$

5.15.8.9.2 Hydroformylation of 2-ethyl-5-(prop-1-enyl)thiophene (**LXIV$_b$**) and 2-allil-5-ethylthiophene (**LXV$_b$**) by homemade 0,18 % Rh/Al_2O_3

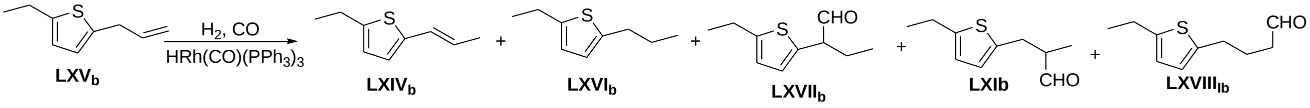


For example, here the procedure adopted for the experiment 7 reported in Table 3.9.13, paragraph 3.9.9 of results and discussions.

In a Schlenk tube, 0.010 g of homemade 0.18 % Rh/Al_2O_3 (**LXV$_b$**/catalyst molar ratio 500/1), 0.013 g (0.087 mmol) of **LXV$_b$** and 10 mL of toluene were introduced under nitrogen. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with cooled to room temperature and the residual gases released. The organic phase was then analyzed by GC and GC–MS. After analysis 99 % conversion of **LXV$_b$** was detected (5 % **LXIV$_b$**, 3 % **LXVII$_b$**, 42 % **LXI$_b$**, 53 % **LXVIII$_b$**, 1 % **LXVI$_b$**).

Adopting the same procedure, the reaction was also performed on **LXIV$_b$** as substrate under the conditions reported in Table 3.9.12 (run 6). After GC and GC-MS analysis, 79 % conversion of **LIX$_b$** was detected (6% **LXVI$_b$**, 47 % **LXVII$_b$**, 26 % **LXI$_b$**)

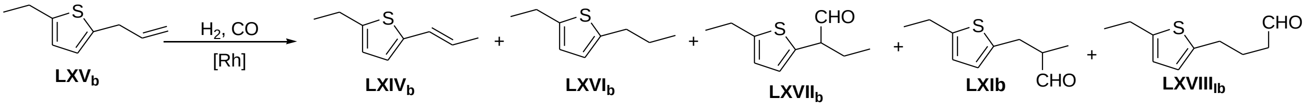
5.15.8.9.3 Hydroformylation of 2-ethyl-5-(prop-1-enyl)thiophene (**LXIV$_b$**) and 2-allil-5-ethylthiophene (**LXV$_b$**) catalyzed by HRh(CO)(PPh$_3$)$_3$



For example, here the procedure adopted for the experiment 7 reported in Table 3.9.13, paragraph 3.9.9 of results and discussions.

In a Schlenk tube, 0.0012 g of HRhCO(PPh$_3$)$_3$ (**LXV$_b$**/catalyst molar ratio 1000/1), 0.195 g (1.3 mmol) of **LXV$_b$** and 4 mL of toluene were placed under nitrogen. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 7 MPa of syngas (CO/H$_2$=1) and heated at 80°C for 20 hours under stirring. The reactor was then cooled to room temperature and the residual gases released. The crude mixture was then analyzed by GC and GC-MS. 99 % Conversion of **LXV$_b$** was detected: 42 % **LXI$_b$**, 53 % **LXVIII$_b$**, 4 % **LXVI$_b$**.

5.15.8.9.4 Hydroformylation of 2-ethyl-5-(prop-1-enyl)thiophene (**LXV$_b$**) and 2-allil-5-ethylthiophene (**LXIV$_b$**) catalyzed by Rh(CO)$_2$(acac)/TPPTS



For example, here the procedure adopted for the experiment 8 reported in Table 3.9.13, paragraph 3.9.9 of results an discussions is reported.

In a Schlenk tube, 0.003 g of Rh(CO)$_2$(acac) (**LXV$_b$**/catalyst molar ratio 1000/1), 0.004 g (0.007 mmol) of TPPTS and 2 mL of water were introduced under nitrogen and stirred for 10 minutes. Subsequently, 0.195 g (0.0013 mol) of **LXV$_b$** and 2 mL of toluene were added. The Schlenk tube was then transferred into a 150 mL stainless steel autoclave under nitrogen, pressurized with 8 MPa of syngas (CO/H$_2$=1) and heated at 80°C for 24 hours under mixing. The reactor was then cooled to room temperature and the residual gases released. Diethyl ether was added and the organic phase was separated from the aqueous phase, than dried on Na$_2$SO$_4$ and analyzed by GC and GC-MS. After analysis 94 % conversion of **LXV$_b$** was detected (6 % **LXIV$_b$**, 35 % **LXI$_b$**, 41 % **LXVIII$_b$,** 6 % **LXVI$_b$**) The recovered aqueous phase was recycled by adopting the above described procedure.

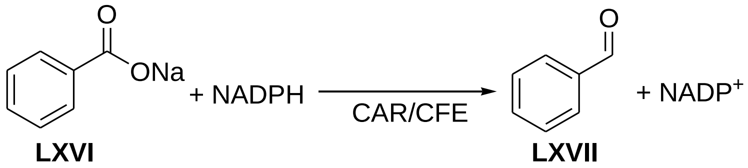
Adopting the same procedure, the reaction was also performed on **LXIV$_b$** as substrate under the conditions reported in Table 3.9.12 (experiment 9).

After GC and GC-MS analysis, 27 % conversion of substrate **LXIV$_b$** was detected (1 % **LXVI$_b$**, 15 % **LXVII$_b$** 11 % **LXI$_b$**).

5.15.9. Application of CAR Enzyme

5.15.9.1. Activity tests of CAR enzyme contained into CFE (Cell Free Extract) and pure CAR enzyme on sodium benzoate (LXVI) and 3-phenylpropionic acid (XLIV)

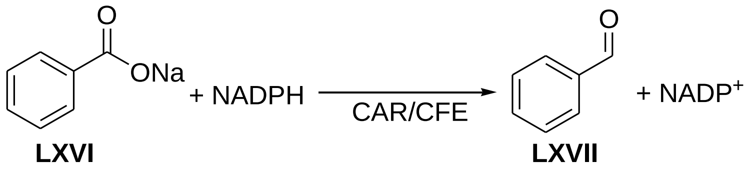
For example, here the procedure adopted with CFE and sodium benzoate (**LXVI**) is reported.



All concentrated solution must be freshly prepared and diluted in HEPES 50 mM buffer at pH = 7.2. In a quartz cuvette with 1 cm of thickness, 100 μL of 20 mM solution of **LXVI**, 100 μL of 100 mM $MgCl_2$ solution, 100 μL of 10 mM ATP solution and 100 μL of 2.5 mM NADPH solution were introduced. After mixing, 500 μL of 50 mM HEPES buffer at pH = 7.2 and 100 μL of Cell Free Extract were introduced. The final concentration of materials were: 2 mM of **LXVI**, 10 mM of $MgCl_2$, 1 mM of ATP, 0.25 mM of NADPH, CFE 10 % v/v. The mixture was immediately mixed and the measurement of absorption at 340 nm was started. The measurement was stopped when the value of absorption was stable.

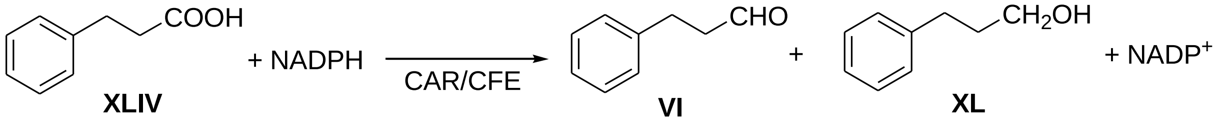
Adopting the same procedure the activity of pure CAR enzyme towards sodium benzoate (**LXVI**) was also checked. In this case, 100 μL of pure solution of CAR enzyme was used. The same procedure was also applied in order to determine the activity of CAR into the CFE and in pure form toward 3-phenylpropionic acid (**XLIV**). In these last case a 20 mM solution of **XLIV** was prepared. Furthermore, the activity of CAR was also determined toward both substrates by using 2.5 mM solution of NADP as cofactor rather than NADPH.

5.15.9.2 Reduction of sodium benzoate (LXVI) by using CFE



In a 50 mL test tube with conical bottom, 0.0086 g (0.06 mmol) of **LXVI**, 0.054 g (0.06 mmol) of NADPH, 0.033 g (0.06 mmol) of ATP, 0.020 g (0.06 mmol) of $MgCl_2$, 10 mL of 50 mM HEPES buffer at pH = 7.2 and 100 μL of CFE were introduced. The reaction was mixed with rolling tube at room temperature for 21 h. After 5 h, 200 μL of mixture were sampled, acidified with 1 drop of 37 % HCl and extracted with 1 mL of diethyl ether. The organic extract was analyzed by GC-MS and it was found that conversion of **LXVI** was 67 % (45 % **LXVII**, 22 % **LXVIII**).

5.15.9.3 Reduction of 3-phenylpropionic acid (XLIV) by using CFE



In a 50 mL test tube with conical bottom, 0.010 g (0.06 mmol) of **XLIV**, 0.054 g (0.06 mmol) of NADPH, 0.033 g (0.06 mmol) of ATP, 0.020 g (0.06 mmol) of $MgCl_2$, 10 mL of 50 mM HEPES buffer at pH = 7.2 and 100 μL of CFE were introduced. The reaction was mixed with rolling tube at room temperature for 21 h. After 5 h, 200 μL of mixture were sampled, acidified with 1 drop of 37 % HCl and extracted with 1 mL of diethyl ether. The organic extract was analyzed by GC-MS and it was found that conversion of **XLIV** was 65 % (62 % **VI**, 3 % **XL**). After 21 h, the same conversion was found.

By adopting the same procedure, the reaction was also performed by using a mixture of HEPES/MTBE 8/2 as solvent (run 2, Table 3.11.3, paragraph 3.11.3) and by using propan-2-ol as sacrificial substrate (run 3, Table 3.11.3, paragraph 3.11.3).

The reaction was also performed for 24 h at 25 °C with 0.01 g (0.06 mmol) of **XLIV**, 0.044g (0.06 mmol) of NADP, 0.037 g (0.066 mmol) of ATP and 0.1 mmol of $MgCl_2$ (0.020 g) dissolved in 9 mL of HEPES buffer (50 mM, pH=7.2) and 1 mL of CFE extract. After 24 h, only traces of desired aldehyde **VI** were detected. By adopting the same conditions, a catalytic amount of NADH was used

(**XLIV**/NADH molar ratio 10/1, 2.2 mg; 166 μL of formate dehydrogenase (equal to 2.5 unit) 0.02 g of sodium formate). After 24 h traces of desired aldehyde **VI** was observed.

5.15.9.4 Activity check of formate dehydrogenase

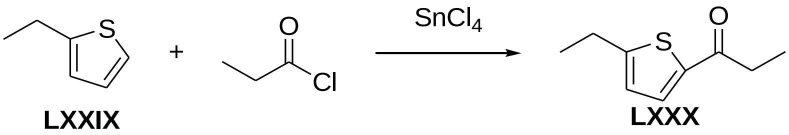
The control was made by adopting the procedure furnished by Sigma-Aldrich[207].

In a quartz cuvette with 1 cm of thickness, 1.1 mL of deionized water, 0.75 μL of 200 mM sodium phosphate buffer pH = 7, 0.75 mL of 200 mM 3-phenylpropionic acid (**XLIV**) solution, 0.3 mL of 10.5 mM NAD solution and 0.1 mL of formate dehydrogenase (1.2.1.2) equal to 0.25-0.50 units/mL were introduced. The value of absorbance at 340 nm was determined for 40 minutes. No change in absorbance was checked. When sodium formate was used, change in absorbance was determined.

5.15.10 Acid catalysis

5.15.10.1. Synthesis of 1-(5-ethylthiophen-2-yl)propan-1-one (LXXX)

5.15.10.1.1 Synthesis of 1-(5-ethylthiophen-2-yl)propan-1-one (**LXXX**) by Friedel-Crafts acylation in the presence of $SnCl_4$



In a two neck 50 ml round bottom flask equipped with magnetic stirrer, 1.12 g (0.01 mol) of 2-ethylthiophene (**LXXXIX**) and 20 mL of dichloromethane at room temperature and under nitrogen were added. The system was cooled at 0°C and then 1.44 mL (0.012 mol) of tin tetrachloride (d = 2.23 g/mL) were added. After 30 minutes, the system was warmed at room temperature and 0.87 mL (0.01 mol) of propionylchloride (d = 1.065 g/mL) were added by a dropping funnel. After two hours, conversion into the desired compound **LXXX** was quantitative. The crude mixture was then put into an Erlenmeyer flask and washed with 50 mL of water and ice for twenty minutes. The mixture was turned from violet to yellow-green. The organic phase was separated from the aqueous phase by a separatory funnel and it was washed three times with 20 mL of diethyl ether.

The organic phases were recovered and dried on Na_2SO_4. After filtration and concentration by rotavapor, the desired product was obtained by vacuum distillation at 6 Pa and 52°C (80 % yield).

GC-MS m/z LXXX: 168 $[M]^+$; 139 $[M; -CH_3CH_2]^+$; 111 $[M; -COCH_2CH_3]^+$
^{1}H-NMR ($CDCl_3$), δ (ppm) LXXX: 7.58 (d, 1H, CH arom.); 6.82 (d, 1H, CH arom.); 2.90 (m, 2H, **CH_2**-CH_3, and CH_3-**CH_2**-CO); 1.35 (t, 3H, ethyl CH_3, J = 7.68); 1.25 (t, 3H, **CH_3**-CH_2-CO, J = 7.32 Hz)

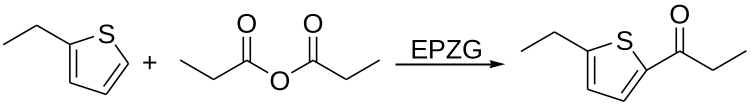
5.15.10.2. Synthesis of 1-(5-ethylthiophen-2-yl)propan-1-one (LXXX) by Friedel-Crafts acylation in the presence of the solid acid catalyst EPZG-10

5.15.10.2.1 Activation of solid acid catalyst Envirocats EPZG

The activation procedure was performed following the supplier's instructions (Contract Chemicals)[194].

The acylation catalyst was activated by means of a Dean-Stark. In a 250 mL round bottom flask 15 g of acylation catalyst and 100 mL of isooctane were introduced. The activation was carried out at reflux of isooctane for ten hours. After this time, the flask containing the catalyst was removed by Dean-Stark under nitrogen. Subsequently, flask was linked at pump and dried under vacuum at 6 Pa. The acylation catalyst was stored under nitrogen.

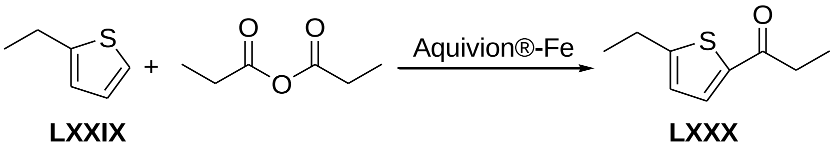
5.15.10.2.2 Synthesis of 1-(5-ethylthiophen-2-yl)propan-1-one (LXXX) by Friedel-Crafts acylation in the presence of the solid acid catalyst EPZG



LXXIX **LXXX**

In a 10 mL two neck round bottom flask equipped with magnetic stirrer and reflux condenser, 1.12 g (0.01 mol) of **LXXIX**, 1.28 ml (0.01 mol) of propionic anhydride (d = 1.01 g/mL) and 0.112 g of EPZG-10 acid catalyst were introduced. The mixture was heated at 120°C for four hours. After this time, 92 % conversion into the desired product **LXXX** and 7 % into an undesired by-product with same molecular weight were detected by GC and GC-MS analysis. The crude mixture was cooled, filtered on a synthered glass filter and distilled under vacuum at 6 Pa and 52°C. The yield of the desired product was 75 %.

5.15.10.3 Synthesis of 1-(5-ethylthiophen-2-yl)propan-1-one (LXXX) by Friedel-Crafts acylation in the presence of Aquivion®-Me



For example, here the procedure of 2-ethylthiophene **LXXIX** acylation catalyzed by Aquivion®-Fe is reported.

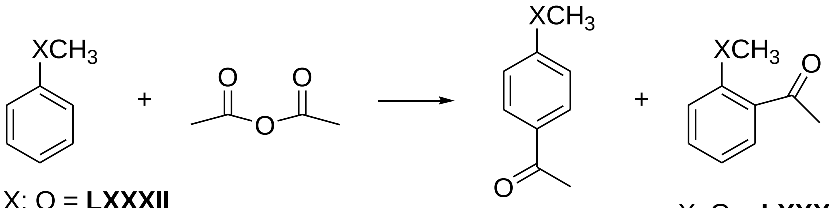
In a 50 mL two neck round bottom flask equipped with magnetic stirrer and reflux condenser, 2 g (0.018 mol) of **LXXIX**, 2.34 mL (0.018 mol) of propionic anhydride (d = 1.01 g/mL), and 1.57 g of Aquivion®-Fe were introduced (**LXXIX**/-R$(SO_3)_3$Fe 32/1 molar ratio). The mixture was heated at 120°C for 3 hours and quantitative conversion into **LXXX** was detected The crude mixture was cooled, filtered on a synthered glass filter and distilled under vacuum at 6 Pa and 52°C. The yield of the desired product **LXXX** was 73 %. The recovered catalyst was re-used by adopting the above described procedure.

Adopting the same above reported procedure, the reaction was also performed in the presence of Aquivion®-In, Aquivion®-Ga and Aquivion® by using also acetic anhydride as acylating agent. The obtained results are reported at paragraph 3.12.2.1.

GC-MS m/z LXXXI: 154 $[M]^+$; -139 $[M; -CH_3]^+$; 111 $[M; -CH_3CO]^+$

^{1}H-NMR ($CDCl_3$), δ (ppm) LXXX: 7.53 (d, 1H, CH arom); 6.85 (d, 1H, CH arom); 2.89 (q, 2H, CH_2); 2,52 (s, 3H, CH_3); 1,34 (t, 3H, CH_3)

5.15.10.4 Acylation of anisole (LXXXII) and thioanisole (LXXXIII) catalyzed by Aquivion®-Me



X: O = **LXXXII**
X: S = **LXXXIII**

X: O = **LXXXIV**
X: S = **LXXXV**

X: O = **LXXXVI**
X: S = **LXXXVII**

For example the acylation of anisole (**LXXXII**) is reported (Table 3.12.2, paragraph 3.12.2.2).
In a 10 mL bottle with screw cap equipped with magnetic stirrer, 2 g (0.019 mol) of anisole (**LXXXII**), 1.80 mL (0.019 mol) of acetic anhydride (d = 1.082 g/mL) and 1.65 g (**LXXXII**/-R$(SO_3)_3$Fe: 32/1 molar ratio) of Aquivion®-Fe were introduced. The mixture was heated at 125°C for 24 hours. The conversion of anisole (**LXXXII**) (52 %) was monitored by GC and GC-MS. 50 % of **LXXXIV** and 2 % of **LXXXVI** were detected. The crude mixture was filtered on a synthered glass filter and purified by silica gel chromatography column using a mixture of n-hexane and diethyl ether (from 8/2 to 5/5) as eluent. It was obtained a little fraction (0.018 g) of pure orto-isomer **LXXXVI**, a mixture of both isomer (0.141 g) and 0.800 g of pure para-isomer **LXXXIV** (yield: 33 %).
Adopting the same procedure the reaction was also performed by using thioanisole (**LXXXIII**) as substrate under the conditions reported in Table 3.12.2 paragraph 3.12.2.2 (the obtained products was purified by chromatography column under the conditions above reported). The reaction was also performed by using different solvent and different Aquivion® salts as reported in Table 3.12.2 paragraph 3.12.2.2. The reaction performed with acetic anhydride as solvent and reactant (run 7, Table 3.12.2) and the reaction performed with toluene as solvent and reactant were carried out in a round bottom flask rather than in the bottle.

GC-MS m/z (LXXXIV): 150 $[M]^+$; 135 $[M; -CH_3]^+$; 107 $[M; -CH_3CO]$; 77 $[M; -CH_3CO\text{-}CH_3O]^+$

^{1}H-NMR (CD_3OD) δ (ppm) LXXXIV: 8.00-7.96 (2H CH arom); 7.50-7.04 (2H, CH arom); 3.87 (s, 3H, CH_3), 2.56 (s, 2H; CH_2)

GC-MS m/z LXXXVI: 150 $[M]^+$; 135 $[M; -CH_3]^+$; 77 $[M; -CH_3CO\text{-}CH_3O]^+$

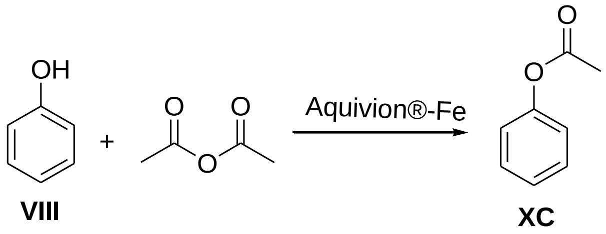
^{1}H-NMR (CD_3OD) δ (ppm) LXXXVI: 7.70-7.50 (2H, CH arom); 7.10-7.00 (d, 2H, CH arom); 3.80 (s, 3H, CH_3), 2.52 (s, 2H; CH_2)

GC-MS m/z LXXXV: 166 $[M]^+$; 151 $[M; -CH_3]^+$; 123 $[M; -CH_3CO]^+$

^{1}H-NMR ($CDCl_3$), δ (ppm) LXXXV: 7.93 (d, 2H, CH arom); 7.35 (d, 2H, CH arom); 2.57 (s, 3H, CH_3), 2.47 (s, 3H; CH_3)

GC-MS m/z LXXXVII: 166 $[M]^+$; 151 $[M; -CH_3]^+$; 123 $[M; -CH_3CO]^+$

5.15.10.5 Acylation of phenol (VIII)

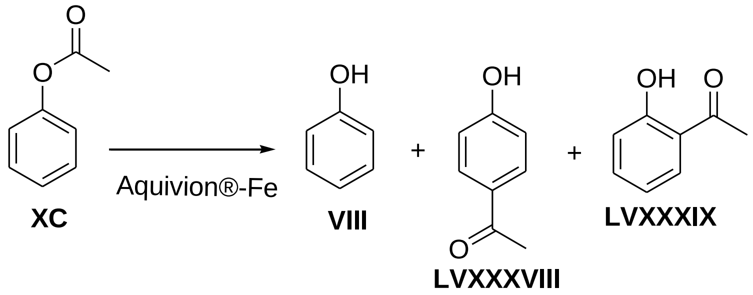


In a 10 mL bottle with screw cap equipped with magnetic stirrer, 2 g (0.021 mol) of **VIII**, 1.98 mL (0.021 mol) of acetic anhydride (d = 1.082 g/mL) and 1.830 g (**VIII**/-$R(SO_3)_3Fe$ molar ratio 32/1) of Aquivion®-Fe were introduced. The mixture was heated at 110°C for 3 hours. Conversion of **VIII** was monitored by GC and GC-MS and 92 % conversion into **XC** was detected. The crude mixture was filtered on a synthered glass filter. After distillation under vacuum (6 MPa, 65°C) the ester **XC** was obtained in 82 % yield.

GC-MS m/z XC: 136 $[M]^+$, 94 $[C_6H_5OH]^+$, 43 $[CH_3CO]^+$

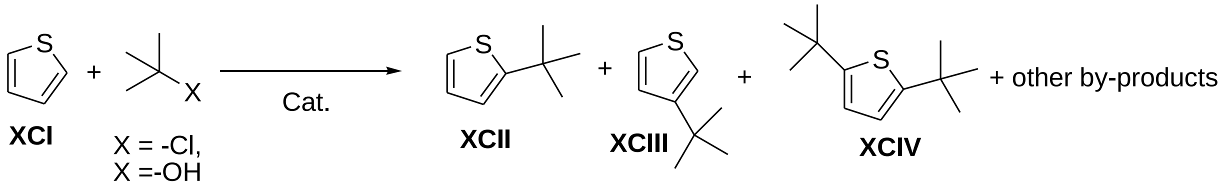
^{1}H-NMR (CD_3OD) δ (ppm) XC: 7.40 (m, CH arom); 7.26 (m, CH arom); 2.25 (s, 3H, CH_3)

5.15.10.6 Fries reaction



In a 10 mL bottle with screw cap equipped with magnetic stirrer, 1 g (0.0073 mol) of **XC** and 0.635 g (**XC**/-R$(SO_3)_3$Fe molar ratio 32/1) of Aquivion®-Fe were introduced. The mixture was heated at 140°C for 7 hours. After this time 43 % conversion into **VIII** was detected by GC and GC-MS. No desired C-acylation product was detected.

5.15.10.7 Thiophene (XCI) alkylation with terbutyl chloride catalyzed by Aquivion®-Fe or with tertbutanol catalyzed by Aquivion®



For example it was reported the run 1 Table 3.9.4, paragraph 3.12.2.4 of results and discussions.
In a 10 mL bottle with screw cap equipped with magnetic stirrer, 2 g (0,024 mol) of **XCI**, 0.222 g (0.0024 mol) of terbutyl chloride and 0.52 g of Aquivion®-Fe (**XCI**/-R$(SO_3)_3$Fe molar ratio 129/1) were introduced. The mixture was heated at 84°C for 16 hours. After this time, the mixture was analyzed by GC and GC-MS and it was found these chromatographic yields: 13 % of **XCII**, 15 % of **XCIII**, 23 % of **XCIV**.

GC-MS m/z XCII=XCIII: 140 $[M]^+$, 125 $[M; -CH_3]^+$, 97 $[M; -C_3H_7]^+$, 85 $[M; -C_4H_9]^+$

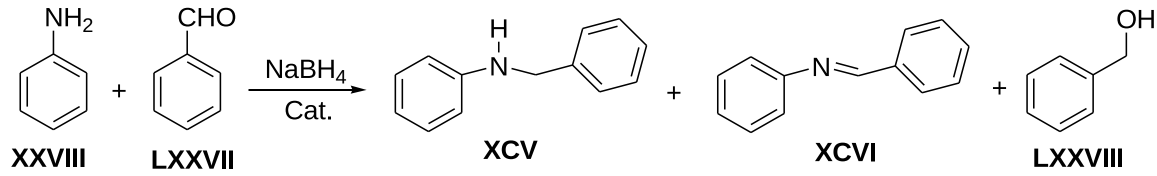
GC-MS m/z XCIV: 196 $[M]^+$, 181 $[M; -CH_3]^+$, 166 $[M; -C_2H_6]^+$, 151 $[M; -C_3H_9]^+$, 97 $[M; -C_7H_{15}]^+$

The mass spectra were attributed by standard injection.

Adopting the same procedure the reaction was also performed by using tertbuthanol as alkylation agent and Aquivion® as catalyst. The obtained results are reported in Table 3.9.4 paragraph 3.12.2.4. The reaction was also performed with isopropylchloride and isopropanol but any reaction did not occur.

5.15.10.8 Reductive amination

5.15.10.8.1 Reductive amination of benzaldehyde (**LXXVIII**) catalyzed by Lewis Acid



In a 50 mL jacketed round bottom flask equipped with magnetic stirrer, 1 g (0.0094 mol) of **LXXVII**, 20 mL of dichloromethane and 0.817 g of Aquivion®-Fe ((**XXVIII-LXXVII**)/-R$(SO_3)_3$Fe molar ratio 32/1) were introduced and the mixture was warmed at 30°C. After 10 minutes, 0.874 g (0.0094 mol) of **XXVIII** and 0.348 g of $NaBH_4$ were introduced. After 3 h, 3 mL of methanol were introduced and immediately a complete conversion of imine into amine was observed. Conversion was monitored by GC and GC-MS. It was found a quantitative conversion of **LXXVII** into 80 % **XCV**, 6 % **XCVI**, 13 % **LXXVIII**. The crude mixture was washed with 20 mL of water and filtered by sintered glass filter. The organic phase was separated from the aqueous phase and the latter extracted three times with 20 mL of dichloromethane. The collected organic phases were dried over Na_2SO_4, concentrated by rotavapor and distilled under vacuum at 6 Pa and 105°C. The yield in the desired product was 60 %.

GC-MS m/z XCVI: 181 $[M]^+$, 104 $[M; -C_6H_5]^+$, 77 $[M; -C_7H_6N]^+$
GC-MS m/z XCV: 183 $[M]^+$, 106 $[M; -C_6H_5]^+$, 77 $[M; -C_7H_8N]^+$
^{1}H-NMR ($CDCl_3$) δ (ppm) XCV: 7.84 (d, 1H, CH arom); 7.46 (d, 1H, CH arom); 3.93 (s, 3H, CH_3); 3.68 (s, 2H; CH_2)
GC-MS m/z LXVIII: 108 $[M]^+$; 90 $[M; -H_2O]^+$; 77 $[M; -CH_2OH]^+$

5.15.10.8.2 Reductive amination in the absence of Lewis Catalyst

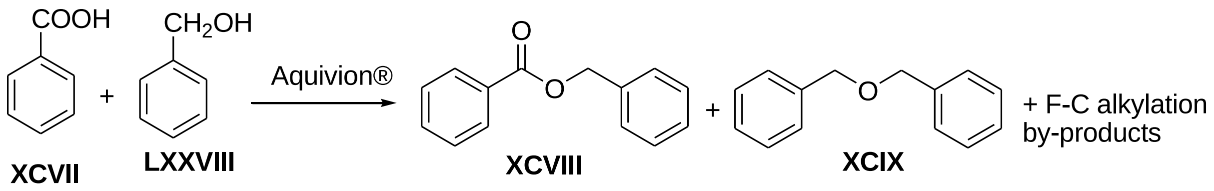
In a 50 mL jacketed round bottom flask equipped with magnetic stirrer, 1 g (0.0094 mol) of benzaldehyde, 20 mL of dichloromethane, 0.874 g (0.0094 mol) of **XXVIII** and 0.348 g of $NaBH_4$ were introduced. After 3 h, 3 mL of methanol were introduced and the conversion was checked by GC and GC-MS. A quantitative conversion of **LXXVII** was determined (53 % **LXXVIII**, 8 % **XCVI**, 38 % **XCV**).

5.15.10.8.3 Synthesis of **XCVI** and subsequently synthesis of **XCV** by subsequently addition of CH_3OH and $NaBH_4$

In a 50 mL jacketed round bottom flask equipped with magnetic stirrer, 0.1 g (0.00094 mol) of **LXVII**, 0.087 g (0.00094 mol) of **XXVIII** and 5 mL of dichloromethane were added. After 10 minutes, 90 % conversion of **LXVII** into intermediate **XCVI** was checked by GC and GC-MS. Then, 0.035 g (0.00094 mol) of $NaBH_4$, and 3 mL of methanol were introduced and quantitative conversion of **XCVI** into the desired **XCV** was observed.

5.15.10.9 Esterification reaction

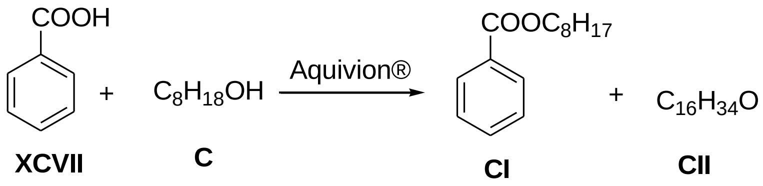
5.15.10.9.1 Reaction between benzoic acid (**XCVII**) and benzyl alcohol (**LXXVIII**) in the presence of Aquivion®



In a two neck, jacketed round bottom flask equipped with magnetic stirrer and reflux condenser, 1 g (0.0082 mol) of **XCVII**, 0.878 g (0.0082 mol) of **LXXVIII**, 10 mL of isooctane and 0.36 g of Aquivion® ((**XCVII-LXXVIII**)/-RSO_3H molar ratio 20/1) were introduced. The mixture was heated at 80°C for 4 h under stirring. Conversion was monitored by GC and GC-MS. Conversion of **LXXVIII** was complete, and it was found 20 % of **XCVIII**, 48 % **XCIX**, 31 % of other Friedel-Crafts alkylation by-products.

GC-MS m/z XCVIII: 212 $[M]^+$; 105 $[M; -C_7H_7]^+$; 91 $[M; -C_7H_5O_2]^+$; 77 $[M; -C_8H_7O_2]^+$

5.15.10.9.2 Reaction between benzoic acid (**XCVII**) and octan-1-ol (**C**) in the presence of Aquivion®

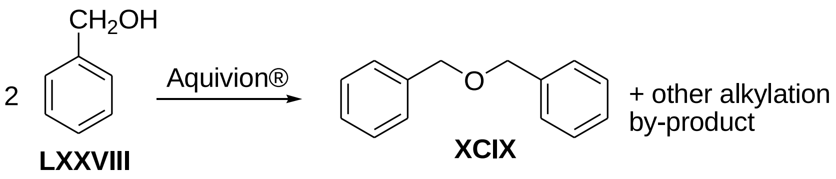


In a two neck jacketed round bottom flask equipped with magnetic stirrer and reflux condenser, 0.200 g (0.0016 mol) of **XCVII**, 0.206 g (0.0016 mol) of **C** , 5 mL of dichloroethane and 0.14 g of Aquivion® ((**XCVII-C**)/-RSO_3H molar ratio 10/1) were introduced. The mixture was heated at 80°C for 24 h under stirring. The determined conversion of **C** was 70 % (68 % **CI**, 2 % in **CII**).

GC-MS m/z CI: 234 $[M]^+$; 105 $[M; -C_8H_{17}O]^+$; 77 $[M; -C_9H_{17}O_2]^+$
GC-MS m/z CII: 112 $[M; -C_8H_{18}O]^+$; 84 $[M; -C_9H_{20}O]^+$

5.15.10.10 Etherification catalyzed by Aquivion®

For example here, is reported the procedure for **LXXVIII** etherification.



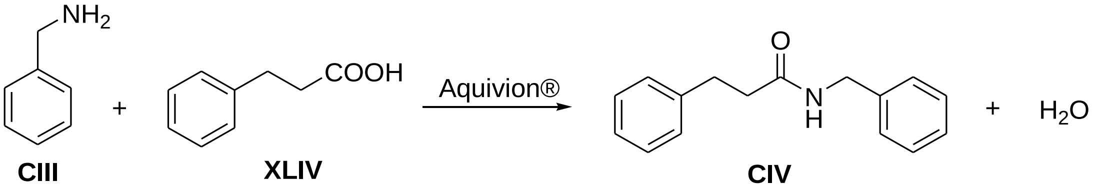
In a two neck jacketed round bottom flask equipped with magnetic stirrer and reflux condenser, 1 g of benzyl alcohol, 10 mL of isooctane and of Aquivion® 0.82 g (**LXXVIII**/-RSO_3H molar ratio 10/1) were introduced. The mixture was heated at 80°C for 3 h under stirring. The conversion was monitored by GC and GC-MS and it was quantitative and 65 % was **XCIX**. The mixture was filtered by sintered glass filter and the organic phase was dried over Na_2SO_4. After filtration and concentration by rotavapor, the desired product **XCIX** was distilled under vacuum at 6 Pa and 85°C to give 40 % yield.

GC-MS m/z XCIX: 170 $[M]^+$; 141 $[M; -COH]^+$; 77 $[M; -C_6H_5O]^+$

^{1}H-NMR ($CDCl_3$) δ (ppm) XCIX: 7.42 (m, CH arom); 4.60 (s, 4H, CH_2)

Adopting the same procedure the reaction was also performed by using octan-1-ol (**C**) as substrate, but after 24 only 2 % conversion into **CII** was found.

5.15.10.11 Amidation



In a two neck, jacketed round bottom flask equipped with magnetic stirrer and reflux condenser, 2g (0.013 mol) of **XLIV**, 1.2 g (0.013 mol) of **CIII**, 20 mL of toluene and 1.16 of Aquivion® g ((**CIII=XLIV**)/-RSO_3H molar ratio 10/1) were introduced. The mixture was heated at 110°C for 16 h under stirring. The conversion was determined by GC and GC-MS. The mixture was then filtered on a synthered glass filter, and the organic phase was washed with a solution of Na_2CO_3. The organic phase was dried on Na_2SO_4, filtered and dried under vacuum. The desired product was obtained in 85 % yield. The reaction was also performed by using Aquivion®-Fe instead of Aquivion® at 60°C, but after 24 h, only 6 % of the desired amide **CIV** was found.

By adopting the above procedure, the reaction was also performed in the absence of catalyst, and the desired product **CIV** was obtained in 82 % yield.

GC-MS m/z CIV: 239 $[M]^+$; 148 $[M; -C_7H_7]^+$; 107 $[M; -C_9H_8O]^+$; 91 $[M; -C_9H_{10}NO]^+$

^{1}H-NMR ($CDCl_3$) δ (ppm) CIV: 7.40-7.00 (m, CH arom); 4.40 (d, 2H, CH_2); 2.72 (t, 2H, CH_2), 2.54 (t, 2H, CH_2)

The reaction was investigated also by using diisopropilamine rather than benzylamine (**CIII**) working from 40 to 80°C, in the presence of Aquivion® but after 24 h no reaction was detected.

6. ACKNOWLEDGEMENT

I thank in particular prof. Stefano Paganelli and prof. Oreste Piccolo for useful advise and availability, Dr. Sperni for her valuable assistance in the GC-MS analysis, Prof. Baldi and Michele Gallo for their cooperation during the preparation of biogenerated catalysts, Prof. Onida and Prof. Fiorilli of Politecnico di Torino for the analysis of heterogeneous catalysts, Prof. Vaccaro of Università di Perugia for the TEM analysis, Prof. Armelao of Università di Padova for the XPS analysis, Dr. Oldani and Solvay Specialty Polymers for Aquivion® sample and for analysis on Aquvion®-Fe and Aquivion®-Ga.

7. REFERENCES

---

[1] P. Pollak, Products, in Fine Chemicals: *The industry and the Business,* 2nd editon, John Wiley & Sons, Hoboken, NJ, USA, 2011

[2] P.T. Anastas, J.C.Warnar (Eds), *Green Chemistry: Theory and Practice*, Oxford University Press, New York, 1998

[3] C.G. Bruntland, *Our common future. The world commission on environmental development,* Oxford university press, Oxford, 1987

[4] D.J.C. Constable, A.D. Curzons, V.L. Cunningham, Green Chem., **4** (2002) 521 and local references

[5] R.A. Sheldon, Pure Appl. Chem., **72** (2000) 1233 and local references

[6] Center for Chemical Process Safety (CCPS), USA, *Inherently safer chemical processes: a life cycle approach*, 2nd ed. John Wiley & Sons, Hoboken, NJ, 2009

[7] M. Eissen, J.O. Metzger, Chem. Eur. J., **8** (2002) 3580

[8] www.ecoscale.org

[9] D.J.C. Constable, C. Jimenez-Gonzales, R.K. Henderson, Org. Proc. Res. Dev., **11** (2007) 133

[10] H.U. Blaser, Catal. Today, **60** (2000) 161

[11] J. Hagen, *Industrial Catalysis a practical approach*, 2nd edition Wiley VCH, Weinheim, 2006

[12] http://goldbook.iupac.org/

[13] C.H. Bartolomew, R.J. Farrauto, *Fundamentals of Industrial Catalytic Processes*, 2nd edition, Wiley and Sons, 2006

[14] A. Sheldon, H. Van Bekkum (Eds.), *Fine chemicals through Heterogeneous catalysis*, Wiley-VCH, Weinheim, 2001

[15] B. Cornils, A. Herrmann, J. Catal., **216** (2003) 23

[16] Prices were in the first week of september 2014. The prices were found on metal-pages.com

[17] N.U. Kumar, B.S. Reddy, V.P. Reddy, R.Bandichhor, Tetrahedron Lett., **53** (2012) 4354

[18] B.Cornils, W.A.Herrmann, I.T. Horvath, W. Leitner, S. Mecking, H. Olivier-Bourbigou, D. Vogt (Eds.), *Multiphase Homogeneous Catalysis*, Wiley-VCH, Weinheim, 2005

[19] P.W.N.M. van Leeuwen, C. Claver (Eds.), *Rhodium Catalyzed Hydroformylation*, Kluver Academic Publishers, Dordrecht, 2000

[20] B. Cornils, W. A Herrmann (Eds.) *Aqueous-Phase Organometallic catalysis*, 2nd ed.; Wiley-VCH, Weinheim, 2004

[21] R.A Sheldon, I. Arends, U. Hanefeld, *Green Chemistry and Catalysis*, Wiley-VCH, Weinheim, 2007

[22] G. Sartori, R. Maggi, Chem. Rev., **106 (**2006) 1077 and local references

---

[23] S. Kobayashi, M. Sugiura, H. Kitagawa, W.L. Lam, Chem. Rev., **102** (2002) 2227

[24] D.L Nelson, M.M Cox, *Lehninger Principles of Biochemistry*, W.H. Freeman and Co., New York 2008

[25] S.L Neidleman, *Aspects of Enzyme Catalysis in Catalysis of Organic Reactions*, Marcel Dekker, New York, 1984

[26] K. Jaeger, Curr. Opin. Biotech., **15** (2004) 269

[27] A. Schmid, F. Hollmann, J.B. Park, B. Buhle, Curr. Opin. Biotech., **13** (2002) 359

[28] S. Panke, M. Wubbolts, Curr. Opin. Biotech., **13** (2002) 77

[29] M.F. Chaplin, C. Bucke, *Enzyme Technology*, Cambridge University Press, New York, 1990

[30] W. Chen, F. Bruhlmann, L. Lee, M. Deshusses, *Whole Cell Catalysis, Encyclopedia of Catalysis,* I.T Horvart ed., John Wiley and Sons, NJ, 2003

[31] B.G Davis, Curr. Opin. Biotech., **14** (2002) 379

[32] J.B. Van Beilen, Z. Li, Curr. Opin. Biotech., **13** (2002) 338

[33] D.R. Yazbeck, A.M. Carlos, S. Hu, J. Tao, Tetrahedron: Asymmetry, **15** (2004) 2757

[34] S.D. Roughley, A.M. Jordan, J. Med. Chem*.,* **54** (2011) 3451

[35] O. Piccolo, A. Verrazzani, US 2005/0033092

[36] F. Baldi, D. Marchetto, D. Zanchettin, E. Sartorato, S. Paganelli, O. Piccolo, Green Chem., **12** (2010) 1405

[37] M.K. Akhtar, N.J. Turner, P.R. Jones, PNAS, **110** (2014) 87

[38] B.Z. Wu, H.Y. Chen, S.J. Wang, C.M. Wai, W. Liao, K. Chiu, Chemosphere, **88** (2012) 757

[39] Y. Rozita, R. Brydson, A. J. Scott, Journal of Physics: Conference Series, **241** (2009) 12096

[40] P. Souza Santos, H. Souza Santos, S.P. Toledo, Materials Research, **3** (2000) 104

[41] J.B. Peri, J. Phys. Chem., **69** (1965) 231

[42] J.B. Peri, J. Phys. Chem., **70** (1969) 3168

[43] C. Yates, J. Chem. Phys., **71** (1979) 3908

[44] G. Bergeret, P. Gallezot, P. Gelin, Y. Ben Taarit, F. Lefebvre, C. Naccache, R.D. Shannon, J. Catal., **104** (1987) 279

[45] O.B. Belskaya, A. Lavrenov, I.G. Danilova, M.O. Kazakov, R.M. Mironensko, A. Likholobov, INTERCH, Open Access Publisher, 2012 (Chapter 7), DOI: 10.5772/36275

[46] F. Baldi, A. Minacci, M. Pepi, A. Scozzafava, Microbiol. Ecol., **36** (2001) 169

[47] S. Leone, C. De Castro, M. Parrilli, F. Baldi, R. Lanzetta, Eur. J. Org. Chem., (2007) 5183

---

[48] F. Baldi, D. Marchetto, D. Battistel, S. Daniele, C. Faleri, C. De Castro, R. Lanzetta, J. Appl. Microbiol., **107** (2009) 1241

[49] I. Arčon, O. Piccolo, S. Paganelli, F. Baldi, Biometals, **25** (2012) 875

[50] L. Armelao, G. Bottaro, R. Campostrini, S. Gialanella, M. Ischia, F. Poli, E. Tondello, Nanotech., **18** (2007) 155606

[51] L. Armelao, D. Belli Dell'Amico, R. Braglia, F. Calderazzo, F. Garbassi, G. Marra, A. Merigo, Dalton Trans., (2009) 5559

[52] W. De Windt, P. Aelterman, W. Verstraete, Environ. Microbiol., **7** (2005) 314

[53] K.B. Narayanan, N. Sakthivel, Adv. Colloid. Interf. Sci., **156** (2010), 1

[54] S. Paganelli, O. Piccolo, F. Baldi, R. Tassini, M. Gallo, G. La Sorella, Appl. Catal. A: Gen., **451** (2013) 144

[55] F. Baldi, A. Minacci, M. Pepi, A. Scozzafava, FEMS Microbiol. Ecol., **36** (2001) 169

[56] J..F Moulder, W.F. Stickle, P.E. Sobol, K.D. Bomben, Handbook of X-Ray Photoelectron Spectroscopy, J. Chastain and R.C. King Jr. eds., Physical Electronics USA, Inc. Chanhassen, Minnesota, 1995

[57] Perkin Elmer Corp., Eden Prairie, MN, 1992; NIST XPS Database (X-Ray Photoelectron Spectroscopy Database 20, Version 3.0, National Institute of Standards and Technology, Gaithersburg, MD; http://srdata.nist.gov/XPS)

[58] T. Hennebel, P. Verhagen, H. Simoen, B. De Gusseme, S.E. Vlaemick, N. Boon, W. Verstraete, Chemosphere, **76** (2009) 1221

[59] J.R. Lloyd, P. Yong, L.E. Macaskie, Appl. Environ. Microbiol., **64** (1998) 4607

[60] L.E. Macaskie, K.M. Bonthrone, P. Yong, D.T. Goddard, Microbiology, **146** (2000) 1855

[61] L. Ben-Dor, I. Felner, Inorg. Chim. Acta, **4** (1970) 49

[62] C. Srilakshmi, N. Lingaiah, P. Nagaraju, P.S. Sai Prasad, K.V. Narayana, A. Martin, B. Lücke, Appl. Catal. A: Gen., **309** (2006) 247

[63] F. Baldi, D. Marchetto, S. Paganelli, O. Piccolo,New Biotech., **29** (2011) 74

[64] a) Franco Baldi, Michele Gallo, Stefano Paganelli, Riccardo Tassini, Oreste Piccolo, Stefano Zambon, Rossano Piazza, Lidia Armelao, Marta Maria Natile, *in preparation* b) XXV Congresso Nazionale della Società Chimica Italiana, Arcavacata di Rende (CS), 7-12 September 2014 ABC 016.

[65] G. Gallo, F. Baldi, G. Renzone, M. Gallo, A. Cordaro, A. Scaloni, A. M. Puglia, Microb. Cell Factories, **11** (2013) 152

---

[66] J. Panpranot, O. Tangjitwattakorn, P. Praserthdam, J.G. Goodwin, Appl. Catal. A: Gen., **292** (2005) 322

[67] T. Maillet, C. Solleau, J. Barbier, D. Duprez, Appl. Catal. B: Environ., **14** (1997) 85

[68] W. Yu, M. D. Porosoff, J. G. Chen, Chem. Rev., **112 (**2012) 5780 and local references

[69] I. Arčon, S. Paganelli, O. Piccolo, M. Gallo, K. Vogel-Mikuš, F. Baldi, in preparation

[70] P. Pontini, Laurea Thesis, A.A. 2013/2014

[71] H.T Uyeda, I.L Medintz, J. K. Jaiswal, S. M. Simon, H. Mattoussi, J. Am Chem. Soc., **127** (2005) 3870

[72] S.Paganelli, O. Piccolo, P. Pontini, R. Tassini, V.D. Rathod, Catal. Today, in press

[73] T. Hedne, O. Samulesson, P. Währborg, H. Wadenvik, Ung KA, A. Ekbom; Drugs **64** (2004) 2315

[74] J.M. Campelo, A. Garcia, D. Luna, J. M. Marinas, React. Kinetic Catal. Lett., **26** (1984) 73

[75] M. Yamaguchi, A. Nitta, R.S. Reddy, M. Hirama, Synlett, (1999) 117

[76] G. Fagassy, L. Hegedus, A. Tungler, A. Lévai, T. Màthé, J. Mol. Catal., **154** (2000) 237

[77] N. Ravasio, F. Zaccheria, P. Allegrini, M. Ercoli, Catal. Today, **121** (2007) 2

[78] D. Cole-Hamilton, R. Tooze (Eds.), *Catalyst Separation, Recovery and Recycling*, Springer, Dordrecht, 2006

[79] E. Kuntz, (Rhône-Poulenc Ind.), Fr. Pat. 2314910, 1975

[80] E.G. Kuntz, Chemtech, **17** (1987) 570

[81] J. Steinreiber, T.R. Ward, Coord. Chem. Rev., **252** (2008) 751

[82] S. Paganelli, M. Marchetti, M. Bianchin, C. Bertucci, J. Mol. Catal. A: Chem., **269** (2007) 234

[83] B. Cornils, Top. Curr. Chem., (1999) 206

[84] W.A Herrmann, C.W Kohlpaintner, Angew. Chem. Int. Ed., **32** (1993) 1524

[85] M. Beller, B. Cornils, C.D Frohning, C.W Kohlpaintner, J. Mol. Catal. A Chem., **104** (1995) 17

[86] P. Panster, S. Wieland, *Immobilization* in “Applied Homogeneous Cataysis with organic compounds”, B. Cornils, W.A Herrmann Ed. VCH Weinheim, **2** (1999) 601

[87] K. Mukhopadhyay, A.B. Mandale, R.V. Chaudhari, Chem. Mater., **15** (2003) 15, 1766

[88] S. Dharmidhikari, M.A. Abraham, J. Supercrit. Fluids, **18** (2000) 1

[89] M. Lenarda, R. Ganzerla, S. Paganelli, L. Storaro, R. Zanoni, J. Mol. Catal. A: Chem., **105** (1996) 117

[90] Michael T. Musser "Cyclohexanol and Cyclohexanone" in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim, 2005.

---

[91] H. Li, J. Liu, H. Li, Mater. Lett., **62** (2007) 297

[92] K. Weissermel, H.J. Arpe, *Industrial Organic Chemistry*, VHC, Weinheim, 1993

[93] S. Scirè, S. Minicò, C. Crisafulli, Appl. Catal. A: Gen., **235** (2002) 21 and loc. references

[94] J.F. Zhu, G.H. Tao, H-Y Liu, L. He, Q.H. Sun, H.C. Liu, Green Chem., **16** (2014) 2664

[95] A.L. Maksimov, S. N. Kuklin, Yu. S. Kardasheva, E. A. Karakhanov; Petroleum Chem., **53** (2013) 157

[96] M. Ohde, H. Ohde, C.M. Wai, Chem. Commun., (2002), 2388

[97] G. Neri, A.M Visco, A. Donato, C. Milone, M. Malentacchi, G. Gubitosa, Appl. Catal. A: Gen., **110** (1994) 49

[98] N. Mahata, V. Vishwanathan, Catal. Today, **49** (1999) 65

[99] J.R. Dojlido, G.A. Best, in E. Horwood (Ed.), *Chemistry of Water and Water Pollution*, Prentice Hall Inc., Englewood Cliffs, NJ, 1993

[100] E. Diaz, A.F. Mohedano, L. Calvo, M.A Gilarranza, J. A Casa, J.J Rodriguez, J. Chem. Eng., **131** (2007) 65

[101] T. Maegawa, A. Akashi, K. Yaguchi, Y. Iwasaki, M. Shigetsura, Y. Monguchi, H. Sajiki**,** J. Chem. Eur., **15** (2009) 6953

[102] J.C.J. Benningshof, R.H. Blaauw, A.E. Van Ginkel, F.P.J.T. Rutjes, J. Fraanje, K. Goubitz, H. Schenk, H. Hiemstra, Chem. Commun., **16** (2000) 1465

[103] D.S Coffey, L.E Overma, F. Stappenbeck, J. Am. Chem. Soc., **122** (2000) 4904.

[104] A. Haudrechy, C. Chassaing, C. Riche, Y. Langlois, Tetrahedron, **56** (2000) 3181

[105] D. Backhaus, Tetrahedron Lett., **41** (2000) 2087

[106] J.C. Raboin, M. Beley, G. Kirsch, Tetrahedron Lett., **41** (2000) 1175

[107] D. Hoeblel, T. Reinerf, A. Schmidt, J. Sol-Gel. Technol., **19** (1997) 115

[108] A. Yoshiura, N. Abe, M. Kudo, Nihon Nohyaku Co. Ltd.,EP 675102, (2000)

[109] J.R. Zoeller, US 2006/49095 (2006)

[110] S. Benetti, R. Romagnoli, Chem. Rev., **95** (1995) 1065

[111] A.L. Lapidus, O.L. Eliseev, T.N. Bondarenko, O.E. Sizan, E.G. Ostapenko, I.P. Beletskaya, Kinet. Catal., **45** (2004) 234

[112] A.L. Lapidus, O.L. Eliseev, T.N. Bondarenko, O.E. Sizan, A.G. Ostapenko, Russ. Chem. Bull. Int. Ed., **50** (2001) 2239

[113] G. Cavinato, L. Toniolo, J. Mol. Catal. A: Chem., **143** (1999) 325

---

[114] R. Tassini, G. La Sorella, D. Montin, S. Paganelli, F. Baldi, O. Piccolo, Chimica e Industria, **9** (2012) 157

[115] I. Pryjomska-Ray, Topics in Catalysis, **40** (2006) 173

[116] T. Hideo, H. Takashi, T. Oshiuki, K. Tagagy, NIPPON NOHYAKU CO. LTD. J20002226428 (2002)

[117] O. Piccolo, D. Montin, S. Paganelli, L. Filippini, M. Gusmeroli, M. Riservato, XVII congresso Nazionale di Chimica Industriale, Genova 2005, CATP31

[118] R. Navon, S. Eldad, K. Mackenzie, F. D. Kopinke, Appl. Catal. B: Environ., **119-120** (2012) 241 and local references

[119] Y. Monguchi, S. Ishihara, M. Ido, M. Nikawa, K. Kamiya, H. Nagase, Y. Sawama, H. Saji, Org. Proc. Res. Dev., **14** (2010) 1140

[120] H. Hildebrand, K. Mackenzie, F.D. Kopinke, Appl. Catal. B: Environ., **91** (2009) 389

[121] F.D. Kopinke, D. Angels-Wedler, D. Fritsch, K. Mackenzie, Appl. Catal. B: Environ., **96** (2010) 323

[122] E. Diaz, A.F. Mohedano, J.A. Casas, L. Calvo, M.A. Gilarranz, J.J. Rodriguez, Appl. Catal. B: Environ., **106** (2011) 469

[123] B.P. Chaplin, M. Reinhard, W.F. Schneider, C. Schüth, J.R. Shapley, T. J. Strathmann, C.J. Werth, Environ. Sci. Technol., **46** (2012) 3655

[124] B.T. Meshesha, R.J. Chimentão, F. Medina, J.E. Suerras, Y. Cesteros, P. Salagre, F. Figueras, Appl. Catal., B: Environ., **87** (2009) 70

[125] C. Schüth, M. Reinhard, Appl. Catal. B: Environ., **18** (1998) 215

[126] E.S. Lokteva, E.V. Golubina, S.A. Kachevsky, A.O. Rurakulova, V.V. Lunin, P. Tundo, Pure Appl. Chem., **79** (2007) 1905

[127] V.S. Baxter-Plant, I.P. Mikheeko, L.E. Macaskie, Biodegradation, **14** (2003) 83

[128] S. De Corte, T. Hennebel, B. De Gusseme, W. Verstraete, N. Boon, Microbial Biotech. **5** (2012) 5

[129] J.R. Kosak, *Catalysis of organic reactions*, Marcel Dekker ed., New York 1984

[130] R.S. Downing, P.J. Kumler, H. van Bekkum, Catal. Today, **37** (1997) 121

[131] Z. Zhao, H. Yang, Y. Li, X. Guo, Green Chem., **16** (2014) 1274 and local references

[132] F.A. Westerhaus, R.V. Jagadesh, G. Wienhöfer, M.M. Pohl, J. Radnik, A.E. Surkus, J. Rabeah, K. Junge, H. Junge, M. Nielsen, A. Brückner, M. Beller, Nat. Chem., **5** (2013) 537

[133] P.P. Sarmah, D.K. Dutta, Green Chem., **14** (2012) 1086

[134] C.V. Rode, R.V. Chaudhari, Ind. Eng. Chem. Res., **33** (1994) 1645

[135] R.A. Surrey, H.F. Hammer, J. Am. Chem. Soc., **68** (1946) 113

---

[136] H. Greenfield, F.S. Dovell, J. Org. Chem., **32** (1967) 3670

[137] J. M. Grosselin, C. Mercier, G. Allmag, F. Grass, Organometallics, **10** (1991) 2126

[138] T. Dwars, G. Oehme, Adv. Synth. Catal., **344** (2002) 239

[139] B. Chen, U. Dingerdissen, J.G.E. Krauter, H.G.J. Lansink Rotgerink, K. Mobus, D.J. Ostgard, P. Panster, T.H. Riermeier, S. Seebald, T. Tacke, H. Trauthwein, Appl. Catal. A: Gen., **280** (2005) 17

[140] H. Gulyàs, A.C. Bényei, J. Bakos, Inorg. Chim. Acta, **357** (2004) 3094

[141] G. Neri, G. Rizzo, L. De Luca, A. Donato, M.G. Mugolino, R. Pietropaolo, React. Kinet. Catal. Lett., **93** (2008) 193

[142] G. Papp, J. Elek; L. Nádasdi, G. Laurenczy; F. Joó, Adv. Synth. Catal., **345** (2003) 172

[143] K.C. Tin; N.B. Wong; R. Li; Y.Z. Li; J.Y. Hu; X.J. Li; J. Mol. Catal. A: Chem., **137** (1999) 121

[144] P. Witte, BASF Corporation, EP 2082805 A1 (2009)

[145] P. Witte, private communication

[146] www.reaxa.com

[147] L. Turin, Flexitral, US 7342042, (2008)

[148] R. Tassini, Laurea Thesis A.A. 2010/2011

[149] L. Cardinali, Laurea Thesis A.A. 2008/2009

[150] M.R. Detty, D.S. Hays, Heterocycles, **40** (1995) 925

[151] N.A. Bumagin, A.N. Kasatkin, I.P. Beletskaya, Seriya Khimicheskaya, **8** (1984), 1858

[152] R.V. Hoffman, R.D. Bishop, P.M. Fitch, R. Hardenstein, J. Org. Chem., **45** (1980) 917

[153] P. Lulinski, L. Skulski, Bull. Chem. Soc. Jpn., **73** (2000) 951

[154] R.M. Kellog, A.P. Schaap, E. T Harper, H. Wynber, J. Org. Chem, **33** (1968) 2902

[155] A. Podgoršek, S. Stavber, M. Zupan, J. Iskra, Green Chem., **9** (2007) 1212

[156] R.J. Lee, H.K. Chao, C.E. Song, Bull. Korean Chem. Soc., **23** (2000) 773

[157] C.Y. Hopkins, M.J. Chisholm, Can. J. Res. B, **24** (1946) 208

[158] H. Nakamura, Y. Tomonaga, K. Miyata, M. Uchida, Y. Terao, Environ. Sci. Tech., **41 (**2007) 2190

[159] R. Norman, J.M. Coxon, *Principi di Sintesi Organica*, Piccin Nuova Libraria ed., Padova, 1997

[160] A. Schoenberg; R.F. Heck, J. Am. Chem. Soc., **96** (1974) 7761

[161] V.P. Baillargeon, J.K. Stille, J. Am. Chem. Soc., **108** (1986) 452

[162] I. Pri-Bar, O. Buchman, J. Org. Chem., **49** (1984) 4009

[163] B.L. Ashfield, C.F.J. Barnard, Org. Proc. Dev., **11** (2007) 39

[164] A. Brennfuhrer, H. Neumann, M. Beller, Angew. Chem. Int. Ed., **48** (2009) 4114

---

[165] K. Natte, A. Dumrath, H. Neumann, M. Beller, Ang. Chem. Int. Ed., **53 (**2014) in press

[166] T. Shibamoto, J. Agric. Food Chem., **28** (1980) 237

[167] D.S. Mottram, I.C.C. Nobrega, J. Agric. Food. Chem., **50** (2002) 4080

[168] G. Biasuzzi, Laurea Thesis, A.A. 2008/2009

[169] R.R. Dykstra, "Hexamethylphosphoric Triamide" *Encyclopedia of Reagents for Organic Synthesis*, John Wiley & Sons, 2001

[170] http://nj.gov/health/eoh/rtkweb/documents/fs/0759.pdf

[171] S. Klaus, H. Neumann, A. Zapf, D. Strübing, S. Hübner, J. Almena, T. Riermeier, P. Groß, M. Sarich, W.R. Krahnert, K. Rossen, M. Beller, Angew. Chem. Int. Ed., **45** (2006) 154

[172] D. Enders, M. Backers, Tetrahedron: asymmetry **15** (2004) 1813

[173] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, Vogel's Textbook, 5th edition, Pearson ed. 2006

[174] C. Stückler, N.J. Müller, C.K. Winkler, S.M. Glück, K. Gruber, G. Steinkellner, K. Faber, Dalton Trans., **39** (2010) 8472

[175] A. He, T. Li, L. Daniels, I. Fotheringham, J.P.N. Rosazza; Appl Environ. Microbiol., **70** (2004) 1874

[176] P. Venkitasubramanian, L. Daniels, J.P.N Rosazza, J. Biol.Chem., **282** (2007) 478

[177] P. Venkitasubramanian, L. Daniels, J.P.N Rosazza , Biocatalytic Reduction of Carboxylic Acids: Mechanism and Application, cap. V, in *Biocatalysis in the Pharmaceutical and Biotechnology Industries*, R.N. Patel ed. CRC Press, 2007 and local references

[178] S. Serra, C. Fuganti, E. Brenna, Trends in Biotechnology, **23** (2005) 193

[179] Y. Ni, P.L. Hagedoorn, J.H. Xu, I.W.C.E. Arends, F. Hollmann, Chem. Comm., **48** (2012) 12056

[180] N. Kato, E-H. Joung, H-C. Yank, M. Masuda, M. Shimao, H. Yamaze, Agric. Biol. Chem., **55** (1991) 757

[181] K. Faber, *Biotransformation in Organic Chemistry*, Springer, Berlin, 2011

[182] J.F Hainfiel, W. Liu, C.M.R. Halsey, P. Freimuth, R.D. Powell, Journal of structural biology, **127** (1999) 185

[183] G.A. Olah, P.S. Iyer, G.K. Prakash, Synthesis, (1986) 513

[184] G.A. Olah, G.K.S. Prakash, A. Molnar, J. Sommer, Superacid chemistry, 2nd ed., John Wiley & Sons Inc. 2009

[185] M.A. Harmer, Q. Sun, Appl. Catalys. A: Gen., **221** (2001) 45

[186] A. Molnar, Curr. Org. Chem., **15** (2011) 3928

---

[187] G.K. Surya Prakash, I. Bychinskaya, E.R. Marinez, T. Mathew, G.A. Olah, Catal. Lett., **143** (2013) 303

[188] www.solvayplastics.com

[189] A. Ghielmi, P. Vaccarono, C. Troglia, V. Arcella, J. Power sources, **145** (2005) 108

[190] M.B. Smith, March's advanced organic chemistry: Reactions, Mechanisms, and Structure, 7th Edition. Wiley, 2013

[191] P. Cagniant, P. Cagniant, Bull. Soc. Chim. France, (1952) 713

[192] M. Sy, Ng. Ph. Buu-Hoi, Ng. D. Xuong, J. Chem. Soc., (1954), 1975

[193] B.P. Bandgar, S.P. Kasture, J. Chin. Chem. Soc., **47** (2000) 1243

[194] Product Information of Contract Chemicals, (UK), http://www.contract-chemicals.com/envirocats.html].

[195] O. Piccolo, L. Filippini, L. Tinucci, E. Valoti, A. Citterio, Tetrahedron, **42** (1986) 885

[196] O. Piccolo, L. Filippini, L. Tinucci, E. Valoti, A. Citterio, Helv. Chim. Acta, **67** (1984) 739

[197] R. Hinze, M.C. Laufer, W.F. Hölderich, W. Bonrath, T. Netscher, Catal. Today, **140** (2008) 105

[198] N.M. Cullinane, B.F.R. Edwards, J. Chem. Soc. (1957) 3016

[199] E. Sartorato, Laurea Thesis, A.A. 2009/2010

[200] R.F. Borch, M.D. Bernstein, H. Dupont Durst, J. Am. Chem. Soc., **93** (1971) 2897

[201] V. Rathod, private communication

[202] M.A. Stanescu, R.S. Varma, Tetrahedron Lett., **43** (2002) 7307

[203] C. Grosjean J. Parker, C. Thirsk, A.R. Wright, Org. Process Res. Dev., **16** (2012) 781

[204] B.M. Monks, A.Whiting in P.J. Dunn, K.K. (Mimi) Hii, M.J. Krische, M.T. Williams, (eds.) *Sustainable Catalysis. Challenges and Practices for the Pharmaceutical and Fine Chemical Industries*, Wiley 2013

[205] D.D. Perrin, W.L.F Armarego, Purification of laboratory chemicals, Pergamon press, 3rd ed, Oxford, 1988

[206] F.X. Felpin, E. Fouquet, Chem. Eur. J., **16** (2010) 12440

[207] www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/General_Information/formate_dehydrogenase_ph_70.pdf

---

Estratto per riassunto della tesi di dottorato

Studente: Riccardo Tassini matricola: 816724

Dottorato: Scienze Chimiche

Ciclo: XXVII

Titolo della tesi :Studio di sistemi catalitici innovativi per la sintesi di prodotti della chimica fine (Study of innovative catalytic systems for the synthesis of fine chemicals)

Abstract: The aim of this work of thesis was the synthesis, characterization and use of new catalytic species used in different type of reaction, by using model substrates in order to understand the advantages and limitations, and in the preparation of some possible industrial and commercial interest fine chemicals.

These new catalytic species were then designed, prepared and characterized in collaboration with several Italian and foreign research laboratories.

During this work of thesis, five new catalytic species were obtained: a) heterogeneous catalysts (Pd, Pt and Rh) alumina based, characterized by very low content of precious metal (0.18 to 0.28%), easy preparation and high activity and selectivity; b) Pd and Rh nanostructured bio-generated catalysts with a possible co-presence of ferric oxide, embedded in the polysaccharide matrix and active in aqueous or in organic solvent-water biphasic system. This new catalytic species were obtained in collaboration with the microbiology laboratory of the DSMN Ca' Foscari by using a strain of Klebsiella oxytoca BAS-10 which is capable to produce a particular exopolysaccharide (EPS) able to bind metal nanoparticles if an appropriate metal salt is added during the stationary growth phase of the microorganism; c) Lewis acids heterogeneous catalysts obtained from a simple and innovative method by using perfluorosulphonate ionomer (Aquvion®) d) a water-soluble and recyclable Rh based catalyst obtained by employing cheap bis-thiol ligand; e) a biocatalyst for selectively reducing of carboxylic groups to aldehyde. This biocatalyst was prepared by fermentation of engineered microorganism and subsequently purified. This work was made during the stage in biotechnological company in Scotland.

The main and most significant studied reactions in this project were: hydrogenation of carbon-carbon double bonds, reduction of some functional groups, hydrodehalogenation, carbonylation, hydroformylation, Friedel-Crafts alkylations and acylations and other acid-catalyzed reactions.

The reactions were carried out both on model substrates on both intermediate of industrial interest. For the latter, it was necessary to develop a synthesis because they are not commercially available. The studied catalytic processes were used to obtain some industrial interest products such as aldehydic and ketonic fragrances (Helional^{TM} and Lioral^{TM}, 5-isopropylthiophene-2-carbaldehyde, 2-propionyl-5-ethylthiophene, 3-phenylpropanal), flavorings (5-ethylthiophene-2-carbaldehyde), drugs and intermediates for drugs

(Nabumetone®, m-chloroaniline), intermediates for agrochemicals (aryl-betaketoesters) and intermediates for bulk chemicals such as cyclohexanone.

Therefore, where possible, the performance of these new catalytic species obtained during with this work of thesis were compared, when the metal was the same, each other, and they were also compared with commercial catalyst. In some cases, the activity and selectivity of these new homemade catalyst species resulted superior. Then, the strategies developed during this research work appear suitable for a possible commercial development.

Riassunto: Oggetto di questa tesi di dottorato è stata la sintesi, la caratterizzazione e l'impiego di nuovi catalizzatori da utilizzare su substrati modello per verificarne vantaggi e limiti e nella preparazione di alcuni *fine chemicals* di possibile interesse industriale e commerciale.

Sono stati quindi progettati, preparati e caratterizzati, grazie anche a collaborazioni con diversi laboratori di ricerca italiani e stranieri, nuovi catalizzatori di 5 diverse tipologie: a) catalizzatori eterogenei su allumina a base di Pd, Rh e Pt, caratterizzati da contenuto molto basso di metallo (0,18-0,28 %), di facile preparazione e in genere altamente attivi e selettivi; b) catalizzatori biogenerati e nanostrutturati a base di Pd e Rh, con eventuale co-presenza di ossido ferrico, in matrice polisaccaridica ed attivi in ambiente acquoso o in sistema bifasico acqua–solvente organico, ottenuti in collaborazione con il laboratorio di microbiologia del DSMN di Ca' Foscari utilizzando un ceppo di *Klebsiella oxytoca* BAS-10 che è in grado di produrre un particolare esopolisaccaride (EPS) capace di legare nanoparticelle metalliche se un opportuno sale metallico viene aggiunto durante la fase stazionaria di crescita del microorganismo; c) catalizzatori acidi di Lewis eterogenei da un polimero polifluorosulfonato commerciale ottenuti con una metodica semplice ed innovativa; d) un catalizzatore idrosolubile e riciclabile a base di Rh e di un economico legando bis-tiolico; e) un biocatalizzatore per ridurre selettivamente gruppi carbossilici ad aldeidici, che è stato preparato mediante fermentazione di un microorganismo ingegnerizzato e successivamente purificato durante il periodo trascorso all'estero in una ditta di biotecnologie in Scozia.

Le principali e più significative reazioni studiate durante il presente progetto sono state: idrogenazioni di doppi legami carbonio carbonio, riduzioni di alcuni gruppi funzionali, idrodealogenazioni, carbonilazioni, idroformilazioni, acilazioni ed alchilazioni di Friedel-Crafts ed altre reazioni acido-catalizzate.

Le reazioni sono state effettuate sia su substrati modello sia su intermedi di interesse industriale, per alcuni dei quali è stata necessaria mettere a punto la sintesi, non essendo commercialmente disponibili. I processi catalitici studiati sono stati impiegati per l'ottenimento di alcuni prodotti di interesse industriale quali fragranze aldeidiche e chetoniche (Helional^{TM} e Lioral^{TM}, 5-i.propiltiofen-2-carbaldeide, 2-propionil-5-etiltiofene, aldeide idrocinnamica), aromi (5-etiltiofen-2-carbaldeide), farmaci ed intermedi per farmaci (Nabumetone®, m-cloroanilina), intermedi per agrochimici (aril-betacheto esteri) ed intermedi per bulk chemicals quali il cicloesanone.