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Lignin for nano- and microscaled carrier systems: applications, trends and challenges

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Abstract:	To liberate the society from the dependence on fossil materials it is pivotal to explore components of renewable plant biomass in applications that benefit from their intrinsic biodegradability, safety, and sustainability. Lignin, a byproduct of the pulp and paper

	industry, is a plausible material for carrying various types of cargo in small- and large-scale applications. We review the constraints regarding physical−chemical properties of the lignin source as well as modifications and processing required to render lignins suitable for loading and release of pesticides, pharmaceuticals, and biological macromolecules. We discuss critically the technical challenges, regulatory and toxicological aspects, and future research needed to realize some of the promises that nano- and microscaled lignin materials hold for a sustainable future.
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18 February 2018

Dear *ChemSusChem* Editors and Reviewers,

On behalf of the authors, I would like you to consider our attached manuscript ***Lignin for nano- and microscaled carrier systems: applications, trends and challenges*** for publication as a Review article in *ChemSusChem*.

The past five years have seen a rapid growth in the number of publications related to sustainable lignin-based materials. One of the emerging applications for lignins is controlled release and other carrier systems for biologically active ingredients.

The objective of our review is to provide a timely and critical evaluation of lignin-based carrier systems, with emphasis on technical, safety, and regulatory aspects of nano- and microscaled lignin-based carriers for pharmaceuticals, enzymes, and pesticides. In addition, we offer our perspective on central research focus for the future.

While there are a few reviews on the production and applications of lignin in general, none of the existing ones provides a comprehensive synthesis of the lignin-based carrier systems across different payload types. We expect that the global need for sustainable carrier materials in the aforementioned applications will attract a broad readership for our review paper in *ChemSusChem*.

Thank you in advance for your consideration. Please do not hesitate to contact me if you would like to discuss any aspect of this submission.

Sincerely yours,

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REVIEW

Lignin for nano- and microscaled carrier systems: applications, trends and challenges

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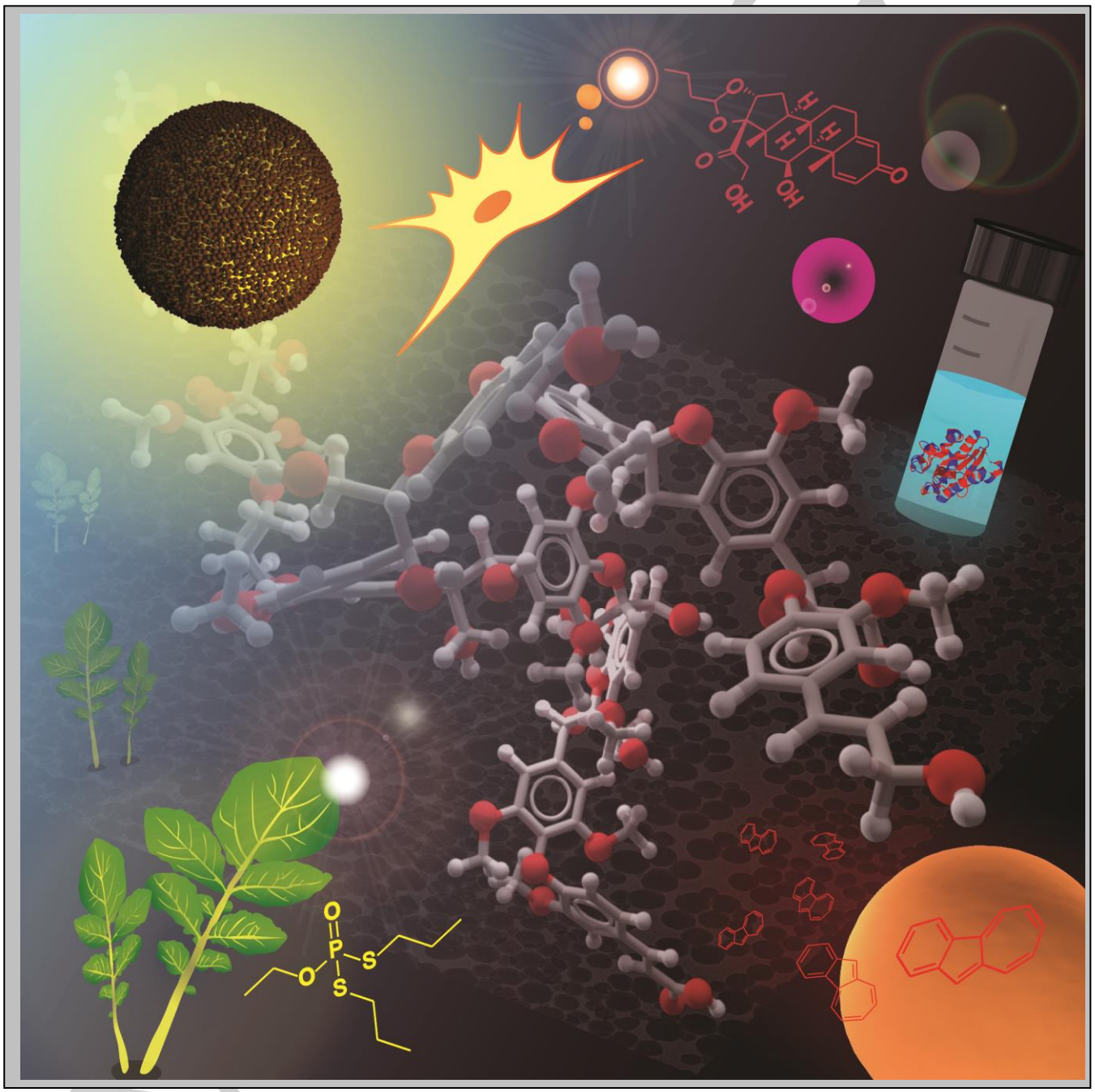

The cover picture shows versatility of lignin as a material for fabrication of carrier systems for active ingredients in nanomedicine, agricultural chemistry, and biocatalysis.

Abstract: To liberate the society from the dependence on fossil materials it is pivotal to explore components of renewable plant biomass in applications that benefit from their intrinsic biodegradability, safety, and sustainability. Lignin, a byproduct of the pulp and paper industry, is a plausible material for carrying various types of cargo in small- and large-scale applications. We review the constraints regarding physical−chemical properties of the lignin source as well as modifications and processing required to render lignins suitable for loading and release of pesticides, pharmaceuticals, and biological macromolecules. We discuss critically the technical challenges, regulatory and toxicological aspects, and future research needed to realize some of the promises that nano- and microscaled lignin materials hold for a sustainable future.

1. Introduction

Lignin is a fascinating biopolymer with huge valorization potential. Besides being the most abundant aromatic renewable biopolymer, its complex structure is responsible for its antioxidant[1–5] and antimicrobial activity.[6–12] Lignins arise from plants' secondary metabolism of 4-hydroxyphenylpropanoid precursors that differ in the number of methoxyl groups at the 3- and 5-positions of the phenolic ring.[13] The structure of a specific lignin material is strongly dependent upon the botanical origin and the isolation process. Especially the harsh thermochemical processes used to separate cellulose from other cell wall components cause fragmentation, functional group eliminations, and process-typical chemical functionalization of lignin.[14–21] In recent years, aside its combustion for energy, isolation of lignin has commenced from industrial kraft pulping processes as well as biorefinery plants producing ethanol as biofuel. This surge in the availability of lignin has been paralleled with development of nano- and microscaled lignin materials,[22–25] and state-of-the-art analytical techniques that give a more detailed understanding of the structural differences posed by various lignins.[26–30]

One of the emerging utilization areas of lignin particles and capsules is their use as carriers for biologically active substances. This current review thus focuses on three application areas for lignin-based carrier systems: plant protection, nanomedicine, and biocatalysis that share some common drivers. Efficient binding/encapsulation of active substances in lignin-based structures is important for all of the above applications, while controlled drug release is emphasized in biomedicine and plant protection.

Current plant protection applications require a vast amount of binders and other materials that facilitate dispersing, shielding from photodegradation, and reducing uncontrolled leaching of agricultural chemicals. According to FAO,[31] global pesticide use was 4.2 kg/ha arable land in 2012, with a total annual consumption of more than 4 Mt. Alongside with the expensive search of new actives, development of new formulations that improve performance of currently approved substances presents many possibilities.[32]

Compared to the vast tonnage of pesticides, the material flows in biomedical applications are far lower, yet with potential for higher added value. Arguably, the currently available amount of lignins suffices to meet the need of biomedical materials. Instead of focusing on the quantity, more attention is required here regarding the assessment of suitability of industrially produced lignins in emerging applications such as in biomedicine. A shortage of literature in this respect does concern the purity and chemical properties of lignin, but also the associated processes that need to be compatible with the application. A comprehensive and critical review on this subject is therefore of pivotal importance. This review takes an in-depth view on lignin-based carriers, including synthesis and surface functionalization used for their realisation. We begin with an outlook on the properties of technical lignins and their suitability in various fields, and continue with the methods used to realise the various carrier structures, to load the active cargo, and to trigger its release. Basing on a critical review of materials that have been used as carriers for pharmaceuticals, biological macromolecules, and pesticides, we identify challenges and opportunities in the current and future applications, before closing with regulatory issues encountered when aiming for nanoscaled materials in marketed applications in the various fields touched in here.

2. Overview of material properties of lignin

The abundance and relatively low cost of lignin in combination with its antioxidant activity[1–5] and UV-shielding properties[2,3,33,34] attract renewed interest in this natural polyphenol. The main lignin types available in relevant quantities are kraft lignin (KL), lignosulfonates (LS), organosolv lignins (OS), and biorefinery lignins that differ notably in their chemical structure, molecular weight, and purity, which in turn determine their solubility and organoleptic qualities. These are important factors influencing the selection of suitable applications in active delivery. For instance, solubility of lignin during and after fabrication of lignin-based carrier materials is of central importance. Depending on their purity, kraft lignins are soluble in aqueous alkali and in some organic solvent mixtures such as aqueous acetone or tetrahydrofuran. Lignosulfonates are soluble in aqueous media over a broad pH range, whereas solubility of organosolv lignins varies largely depending on the pulping process. Biorefineries hold considerable potential to emerge as important sources of industrial lignins,[35] but their solubility properties are unpredictable due to the unsettled process conditions and/or

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variability in feedstock materials. Table 1 gives an overview of lignins most commonly used as raw materials when fabricating carriers for a variety of active ingredients. The selection of lignin type depends on the application category, *i.e.*, purified or fractionated lignins should be considered for biomedicine, whereas less pure preparations are deemed suitable for technical applications such as controlled release of pesticides. However, this quality-safety aspect of lignin has been left with little attention in the literature.

Table 1. Molecular weight and solubility characteristics of common lignins used in fabrication of various carrier materials for active substances.

Lignin source	Type[a]	Isolation process	Typical impurities
Softwood, hardwood	KL	Kraft pulping	Carbohydrates <5%
Softwood, hardwood	LS	Sulfite pulping	Salts
Softwood, hardwood	OS	Ethanol-water organosolv pulping	Carbohydrates, extractives
Annual plants.	SL	Soda pulping	Carbohydrates <10%, silica
Annual plants	HTL	Autohydrolysis or acid-catalyzed pretreatment and extraction	Carbohydrates <10%
Various types of biomass	EHL	Solid fraction recovered after saccharification of pretreated biomass	Carbohydrates >10%

[a] Type of lignin: KL (Kraft lignin), LS (lignosulfonate), OS (ethanol organosolv), SL (soda lignin), HTL (hydrothermal lignin), EHL (enzymatic hydrolysis lignin).

3. Lignin-based materials and methods for active loading

Lignin-based materials can be ordered according to descending particle size in hydrogels, granules, microcapsules, microparticles, nanocapsules, and nanoparticles (NPs). Research output in these categories has increased within the span of the last six years, with the exception of lignin granules (Figure 1). The most extensive growth in the number of publications dealing with lignin nanoparticles (LNPs) reflects strong anticipation for their potential applications. Some authors use the term “colloidal lignin particle” (CLP) interchangeably with LNP; thus the number of non-duplicate publications in these two categories are summed in Figure 1.

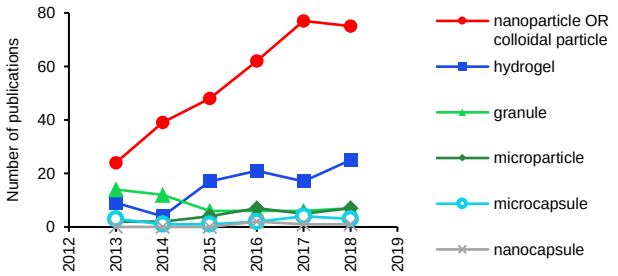

Figure 1. Number of publications returned to the search string “Lignin AND keyword” in the Scopus database (search limited to title, abstract, keywords, duplicates removed).

Fabrication of lignin particles in nano- and microscales have been reviewed recently,[22,23] and we therefore limit this effort to a brief overview of the methods frequently used (Figure 2). The most common method for the preparation of lignin NPs involves solvent exchange by adding a non-solvent into lignin solution, or *vice versa*, causing formation of spherical particles due to the minimization of surface energy (Figure 2a−c).[36] Aqueous and non-aqueous tetrahydrofuran (THF),[37,38] dioxane,[39] dimethylsulfoxide (DMSO),[33,40] acetone[2,33,41] and ethanol[42,43] have been used as lignin solvents, while water is the most commonly used non-solvent to produce spherical particles. In the case of the ethanol-water system, the particle diameter has been reported to increase as the dilution rate decreases (Figure 2c).[42,43] The resulting spherical and spheroidal particles exhibit comparable surface charges to those formed using the regular solvent exchange method. Stable CLP dispersions have been reported at concentrations up to 3 % (w/w) when using ethanol as co-solvent with tetrahydrofuran.[44] CLPs exhibit the lignin-typical antioxidant and UV-protective properties[3] that are useful for instance in sunscreens.[45–49] Aerosol technology is another approach to prepare lignin nano- and microparticles (Figure 2d).[50] In this process, solvent is vaporized from a solution of lignin, forming particles at the hydrophobic solvent-air interface. Emulsions templates represent a common approach to lignin nano- and microcapsules (Figure 2f).[51–55] In addition, synthesis of lignin nanotubes has been conducted using sacrificial aluminum templates.[56]

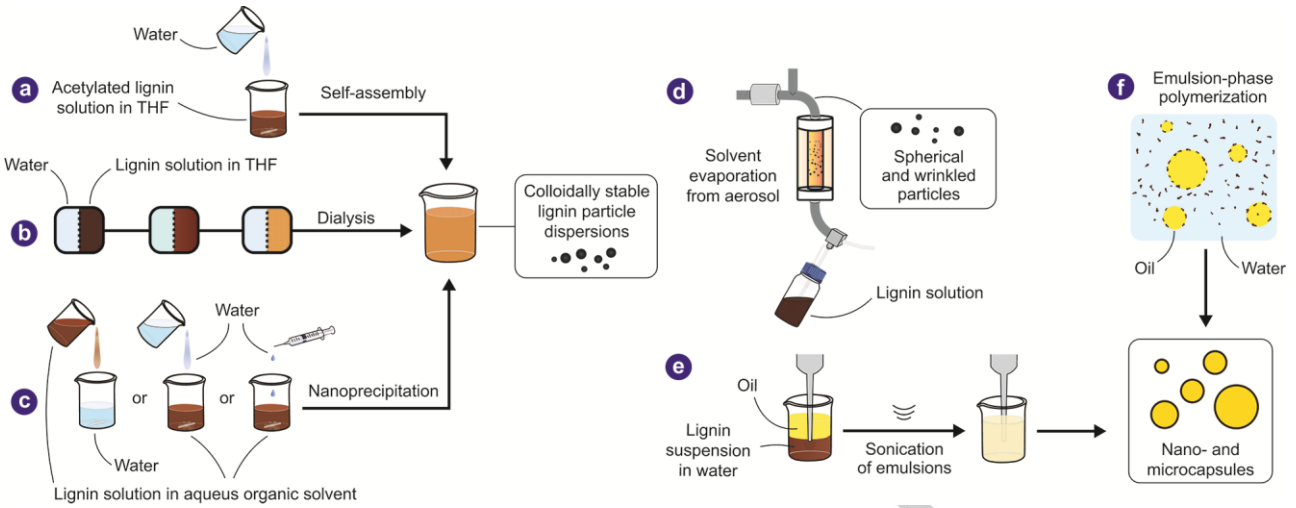

Figure 2. Methods used in fabrication of nano- and microscale lignin materials. Colloidally stable lignin particle dispersions prepared by (a) Adding water into THF solution of acetylated wheat alkali lignin; (b) Dialyzing non-acetylated softwood kraft lignin in THF solution against deionized water, Adapted from Ref.[37] with permission from The Royal Society of Chemistry; (c) Nanoprecipitation by adding water into lignin solution, or vice versa. Common solvents used include ethanol,[1,43] THF,[38] and acetone.[2] (d) Formation of micro- and nanoparticles in an aerosol flow reactor. Adapted with permission from Ref.[50] Copyright (2016) American Chemical Society. (e) Microcapsules formed by ultrasonication of kraft lignin containing a cross-linker. Adapted with permission from Ref.[51] Copyright (2014) American Chemical Society. (f) Nanoparticles, nanocapsules, and porous microparticles by emulsion-phase polymerization and cross-linking. Adapted with permission from Refs.[52,57] Copyright (2016, 2017) American Chemical Society.

Although not present in the final product, it is worth mentioning that lignin has been used as a sacrificial substance on the way to a final material. Tardy *et al.* used kraft and alkali lignins as sacrificial core in the generation of capsules comprised of 'phenol-metal' complexes.[58] In this case, the phenols forming the shell of the capsule after dissolution of the sacrificial lignin core were tannins. The advantage of using lignin as a sacrificial core is that problems normally encountered in the dissolution of sacrificial polystyrene cores, *i.e.*, the use of non-green and / or non-benign solvents like tetrahydrofuran are avoided. Later, Piccinino *et al.* constructed micro- and nanocapsules/nanoparticles using a sacrificial core made of micro-sized beads from inorganic manganese carbonate or organosolv lignin-based nanoparticles and tannic acid and lignosulfonate for coating.[59] The products showed antioxidant activity, UV-shielding properties, and electrochemical responsiveness in form of synergistic effects.

Entrapment, encapsulation, adsorption, and covalent binding are common methods for loading actives into lignin materials (Figure 3). The loading of the cargo may take place during or after the formation of nanoparticles, nanocapsules, etc. Simultaneous loading has been reported by entrapment in LNPs[1,41,60–63] and encapsulation in emulsion polymerization.[53,64,65] Layer-by-layer (LbL) adsorption can be used to assembly actives within polymer multilayers,[66] for instance to increase the amount of loaded active, while reducing its release rate.

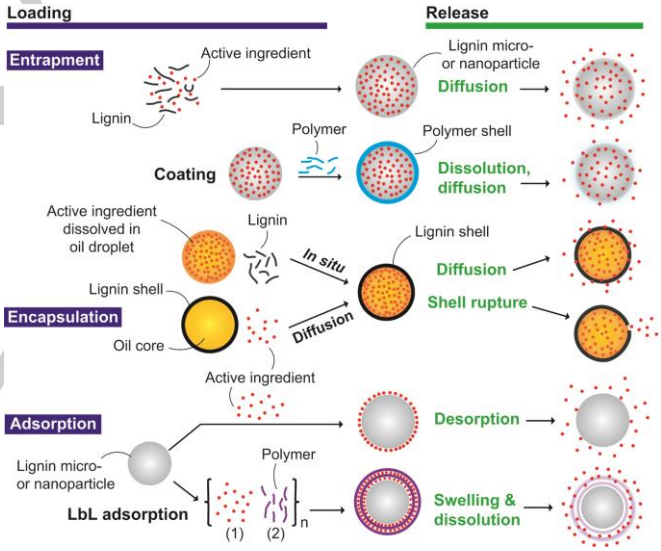

Figure 3. General methods for loading and releasing actives from various particulate and capsule carriers.

4 Entrapment, encapsulation, and adsorption of actives in/on lignin carriers

4.1. Entrapment

Entrapment is by far the most common method to load active cargo in lignin-based materials. The current view is that lignin NPs form by supramolecular assembly of poorly water-soluble molecule domains *via* electric interactions with aromatic rings.[1,36,37] As could be expected from this mechanism, most of the substances entrapped in lignin NPs are low molecular weight compounds with low water-solubility (Figure 4). Pesticides

(herbicides, insecticides, microbicides)[41,67–75] is the largest class of compounds that have been entrapped. Commercial interest in lignin-based carriers is at least partly due to the well-suited intrinsic properties of lignin as a polyphenol. Lignins can (i) provide carriers capable of triggered slow or fast release of pesticides; (ii) shield UV-sensitive or toxic substances; (iii) facilitate dispersion; (iv) prevent unwanted erosion of volatile, eventually toxic actives; and (v) substitute currently used synthetic polymers in these applications.[72,76,77] Additionally, due to the slow rate of biodegradation of lignin, it can contribute to maintaining the soil carbon balance.[78] Besides pesticides, there are a few works that have demonstrated lignin-based materials for enzyme immobilization,[79,80] and delivery of active pharmaceutical ingredients.[1,60,61,81]

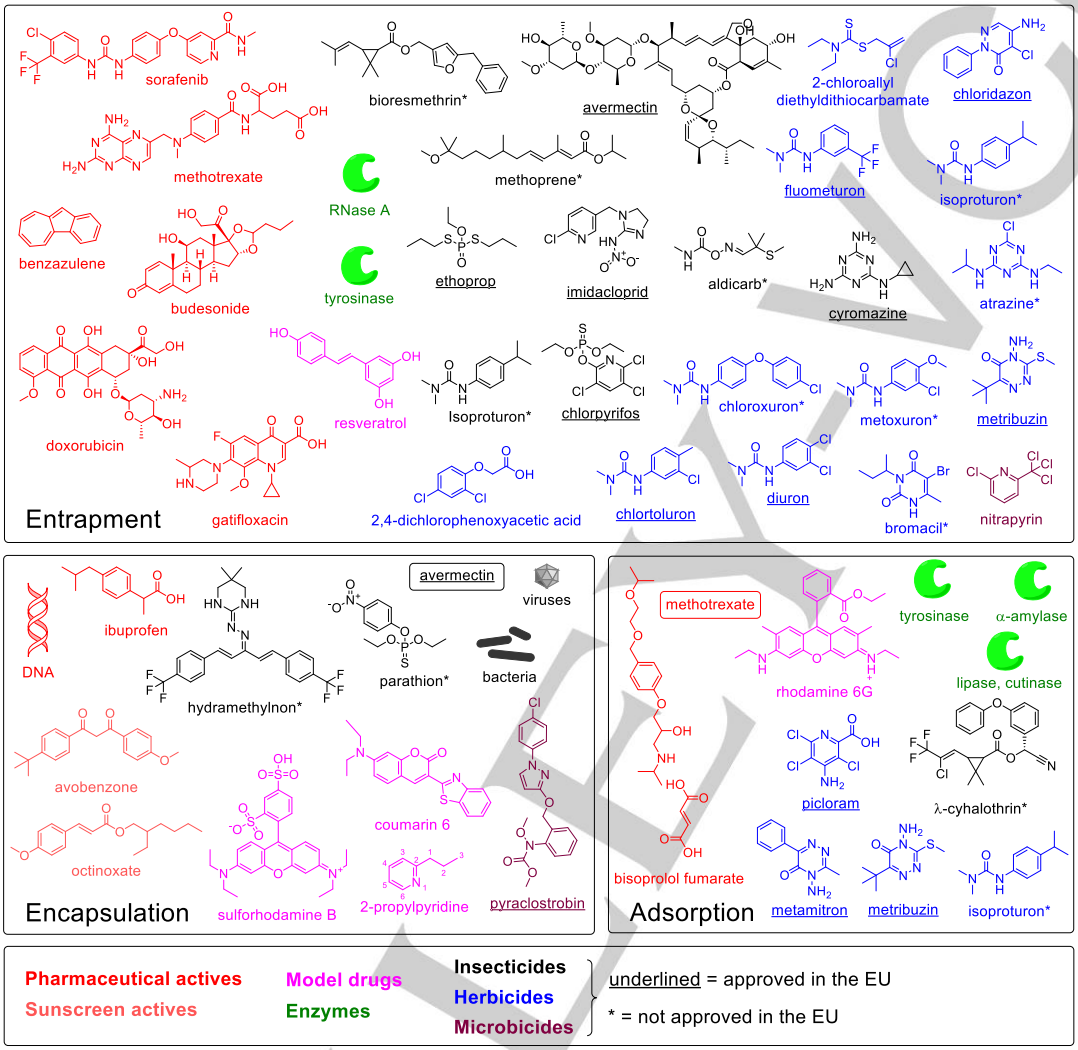

Figure 4. Organic substances and corresponding loading methods onto/into lignin materials. Current approval status of the pesticides were retrieved from ref.[82]

Entrapment efficiency (EE-%) is reported to vary broadly from 4% to >95% depending on the carrier formation process, the lignin carrier morphology and the type of entrapped active ingredient (Table 2). Typical loading capacities (LC) have been less than 20 wt%, with a few exceptions of higher concentrations.[62,71,79,81] However, methods used to analyze the LC and EE-% have not been standardized for lignin particles. A particular challenge needing attention relates to the isolation of the NPs from the aqueous medium that contains the free, soluble active substances. Centrifugation using ultrafiltration membranes has been used to isolate lignin NPs from non-entrapped actives in aqueous phase.[1,79] However, such procedure may not be suitable to all kinds of active substances that may release rapidly during the purification procedure. Further work is required to establish purification methods that remove selectively the non-entrapped active substances, while leaving the entrapped molecules intact in the solid particles. Solvent extraction with water-immiscible solvents that do not disrupt the lignin particles may be one possible route towards a more reliable, standardized methodology.

Table 2. Lignin-based materials used for the entrapment of actives.

Carrier material	Active	EE (%)[a]	LC (wt%)[b]	Ref.
LNPs (WS soda lignin)	Budesonide	35	3.5	[1]
LNPs (SKL)	BZL, SFN	77 BZL, 68 SFN	8 BZL, 7 SFN	[60]
LNPs (AL, Sigma-Aldrich)	Doxorubicin (DOX), gatifloxacin (GFLX)	90 DOX, 5–37 GFLX	47% DOX, 5-27% GFLX[c]	[61]
LNPs (alkali lignin from HT-pretreated corn cobs)	Resveratrol	71–95	19–26	[62]
LNPs (dioxane ligning from subabul stems)	Diuron	74	5.2	[41]
LNPs (OSL)	Tyrosinase	69	12	[80]
LNPs (succinylated SKL)	Benzazulene	50–57	9–11	[63]
Lignin-based complex micelles (alkali lignin)	Ibuprofen	74	46	[81]
Chitosan-LNPs (calcium-LS)	RNase A	61–40	6.6–43	[79]
Lignin-PVA microparticles (spruce ionic lignin, sugarcane bagasse OSL)	Atrazine	39–78	4–15	[67]
Lignin microcapsules (azo-modified poplar alkali lignin)	Avermectin	61	17	[68]
SLS-CTAB microspheres	Avermectin	71	63	[83]
Lignin microcapsules (SKL, SLS)	Nitrapyrin, Chlorpyrifos	>69	6.6	[69]
Cross-linked xanthan/lignin hydrogel (ALS)	Bisoprolol fumarate	n.a.	14–19	[70]
Self-assembled alkyl-modified lignosulfonates	Avermectin	50	57	[71]
Lignin hydrogel (SKL, sulfonated SKL, lignosulfonate)	Ethoprop, methoprene, bioresmethrin	97–100[a]	n.a.	[72]
Lignin/silica hydrogel (Sugarcane bagasse soda lignin)	Methotrexate	n.a.	n.a.	[73]
Dried lignin hydrogel (SKL)	2-chloroallyl diethyldithiocarbamate			[75]
Lignin-CMC hydrogel (SKL)	Aldicarb	4–13	≤3.0	[74]

[a] Entrapment efficiency. [b] Loading capacity in lignin carrier. [c] Calculated from the reported EE% and initial mass ratio of lignin and actives. LNPs, lignin nanoparticles; WS, wheat straw, Benzazulene (BZL), sorafenib (SFN), n.a., not available. [a]: based on mass balance. KL, kraft lignin. SKL, softwood kraft lignin. HT, hydrothermal. OSL, organosolv lignin. LS; lignosulfonate, SLS, sodium lignosulfonate. ALS, ammonium lignosulfonate.

4.1.1 Pesticides

Many pesticides are potent groundwater pollutants, and hence their leaching from soil should be avoided. We note that regulatory status of pesticides has changed in recent years, and almost half of the pesticides shown in Figure 4 are now banned in the EU. Here we visit a few examples of studies involving currently non-banned pesticides in nano- and microscaled lignin materials. Among those pesticides still approved for use is avermectin, a cyclic lactone excreted by the soil bacterium *Streptomyces avermitilis*. In addition to its insecticidal activity, avermectin is used medicinally as an antiparasitic substance.[84] Deng *et al.* demethylated poplar alkali lignin and derivatized it by azo-coupling with diazonium salt of aniline.[68] Co-precipitation of these novel lignins with avermectin from THF solution in water formed loaded NPs that protected avermectin against photodegradation under UV irradiation compared to non-entrapped avermectin. However, the authors did not show photostability of avermectin in regular LNPs. Two recent papers developed alternative lignin-based carriers for avermectin. Entrapment in alkylated lignosulfonate microcapsules protected avermectin from UV irradiation and additionally retarded its release in aqueous ethanol.[71]

Li *et al.* generated lignin-based microspheres through self-assembly. Lignosulfonate and cetyltrimethylammonium bromide (CTAB) self-assembled into spherical particles by dropwise addition of water into their ethanol solution. Exhibiting a reversible aggregation behavior, the material was used for preparing microspheres to encapsulate water-soluble avermectin.[83] Highlightable features of this work comprise a tunable release profile adjustable by varying the ratio between the lignosulfonate and the CTAB. The half-life of UV-sensitive avermectin under UV irradiation could be prolonged for more than seven times once encapsulated. On the other hand, concentration of the avermectin-containing microsphere suspension was only 0.06 wt% and optimization of this important parameter was not shown. Furthermore, though the work of Li *et al.* avoided using covalent modification, all of the above procedures with avermectin used synthetic chemicals, which should be abandoned to realize more environmentally benign approaches.

Herbicides are chemical substances to control weed growth. Chloridazon is a volatile herbicide that was loaded from methanol solution into *a priori* formed microcapsules in lignin gel.[69] After nine days of storage at 55 ºC, 40% of chloridazon was lost due to volatilization, but the remaining amount remained stable for five consecutive days, whereas exposure to water triggered a faster release. Lignin isolated using the acidic dioxane method from subabul stems was used to entrap herbicide diuron by nanoprecipitatation from acetone-water (9:1 v/v) solution containing 1% poly(vinyl alcohol).[41] Release of diuron from the nanoparticles immersed in aqueous buffer solutions increased with increasing pH from 5 to 9, and was significantly slower compared to the dissolution of bulk diuron or commercial diuron formulation.

4.1.1 Pharmaceuticals

Pharmaceuticals and other substances used in biomedical applications represent an increasing group of active cargo. Figueiredo *et al.* entrapped the poorly water-soluble drug sorafenib (SFN) and benzazulene (BZL) as a cytotoxic agent in softwood kraft lignin NPs.[60] Loading capacities of 7–8% were obtained at 68–77% entrapment efficiencies. SFN and BZL contain , thus differing from a water-soluble drug capecitabine that could not be entrapped. The same group reported a 50% entrapment efficiency of BZL in LNPs prepared from succinylated lignin that allowed conjugation of the particles with amine-functionalized polyethylene glycol and cell-penetrating peptides.[63] Sipponen *et al.*[1] entrapped 3.5% budesonide in LNPs prepared from wheat straw soda lignin, which means that less than 300 mg of the nanoformulation would fulfil the daily dosage of this anti-inflammatory corticosteroid drug. Although the EE was only 35%, successful recovery and reuse of the soluble

budesonide was demonstrated. The cytotoxic agent doxorubicin and antibiotic gatifloxacin were dissolved in aqueous p-toluenesulfonate solution with alkaline lignin, and precipitated by adding water, therefore entrapping the actives in the lignin particles.[61] The authors reported a high EE of 90% for doxorubicin, which was higher compared to the maximum of 37% obtained with gatifloxacin. Likely due to its low water-solubility, doxorubicin was released incompletely when the loaded LNPs were immersed in saline solution.

4.1.1 Enzymes

Enzymes are so far the only macromolecules entrapped in lignin-based nano- and microscaled materials. The motivation for enzyme immobilization arises from the need to reuse the water-soluble catalysts, improve their activity, separate products from the catalyst, and application of flow-through reactions instead of batch reactors.[85] The enzyme supports must be stable under the reaction conditions, *i.e.* be inert towards solvents and reagents, and resist shear and thermal stress. The use of low-cost lignin-based materials for immobilization enables disposal of the inactivated biocatalyst by environmentally benign ways such as composting or combustion. Entrapment of RNase A in chitosan-lignosulfonate NPs was achieved by ultrasonication of the components at an oil-water interface.[79] The maximum EE% and loading capacity of RNase A were 61% and 43 wt%, respectively. Different release profiles observed with low and high amounts of the protein cargo indicated its entrapment in the bulk matrix and adsorption on the surfaces. Another type of enzyme entrapped in lignin NP matrix is tyrosinase that catalyzes oxidation of tyrosine and a number of other phenolic substances.[80] The activity-based immobilization yield at a 12 wt% loading capacity was 69%, which are roughly similar values as the ones reported for RNase at comparable loading capacities.[79] As opposed to the physically entrapped enzyme inside LNPs, systematically higher catalytic activity in oxidative conversion of 4-methylphenol was achieved with tyrosinase adsorbed on LNPs.[80]

4.2. Encapsulation

Encapsulation is a strategy to deliver lipophilic drugs that are soluble in the water-immiscible cores of capsules dispersed in aqueous media, and vice versa. The ratio of sphere volume to surface area equals diameter/6, and therefore microcapsules appear more efficient than nanocapsules if compared solely based on the volume available for loading of the active. However, NPs with diameter of around 200 nm have exhibited benefits over larger particles in drug delivery to cancer cells.[60,86,87] The (self-)aggregation tendency of lignins can be exploited in the formation of capsules, *i.e.*, structures comprised per definition of a core-shell construct; shells can be of varying rigidity, cores can be solid or liquid. It is needless to emphasize that systems are preferred that can be obtained without the need of sacrificial elements and / or materials.

Table 3. Lignin-based materials used for the encapsulation of actives.

Carrier material	Active substance	EE (%)[a]	LC (wt%)[b]	Ref.
LNCs (AL)[c]	Coumarin 6	70–90	n.a.	[88]
LNCs (SLS)	Coumarin 6	n.a.	n.a.	[53]
LNCs (SLS)	Sulforhodamine	n.a.	n.a.	[65]
LNPs and LNCs (KL)	2-propylpyridine	n.a.	n.a.	[52]
Hollow LNPs (alkali lignin)[c]	Pyraclostrobin	64–100	2.1–15	[64]
LNC (EHL)	Avobenzone, Octinoxate	98	53	[48]
LMC (alkali lignin)[c]	Coumarin 6	n.a.	n.a.	[51]
Lignin-gelatin-formaldehyde resin (azo-modified LS)	Parathion	n.a.	n.a.	[76]
LMC (SKL)	Hydramethylnon, *Bacillus thuringiensis*, viruses	n.a.	n.a.	[77]
Complex micelles (SKL)	Ibuprofen	74	46	[81]

[a] Entrapment efficiency. [b] Loading capacity in lignin carrier. [c] Alkali lignin from Sigma-Aldrich. LNC, lignin nanocapsule. LMC, lignin microcapsule. KL, kraft lignin. EHL, enzymatic hydrolysis lignin. n.a., not available. LS, lignosulfonate,. SLS, sodium lignosulfonate.

4.2.1 Pesticides and miscellaneous actives

Relying on self-aggregation of lignins alone during the sonication step, recent studies describe lignin microcapsules able to encapsulate hydrophobic actives.[51,88] Starting from an emulsion comprised of a lignin-containing aqueous phase and an active-containing oil phase, the amphiphilic lignin oligomers and polymers arranged at the water-oil interface upon ultrasonication and aggregated under these conditions.[51] Addition of organic crosslinkers like diglycidyl-terminated PEG can serve to strengthen the capsule structure. In addition, the use of ferric chloride as inorganic crosslinker, employing the complexation capabilities of the phenolic OH-groups towards metal ions, serves to strengthen the shell with respect to regular capsules.[88] Hydrophobic actives mixed into the oil prior to capsule generation stay in the internal oil phase. Lignin microcapsules obtained by sonication showed a good shelf-life and a general non-cytotoxicity.[51] These features, together with the ability to only slowly disintegrate when chemical triggers like higher hydronium ion concentrations are used, make them potentially versatile candidates for drug release applications.

Yiamsawas *et al.* applied the opposite strategy for the preparation of their lignin-based nanocontainers.[65] The aqueous lignin solution was emulsified in an organic phase of cyclohexane containing toluene diisocyanate and a surfactant. The polymerization of lignin polyurethane takes place at the water–cyclohexane interface to yield hollow nanocapsules of lignin-based polyurethane. Because of the reaction of toluene diisocyanate with water, urea linkages are formed together with

the target urethane linkages. The encapsulating capacity and stability of the capsules were evaluated using the hydrophilic fluorescent dye sulforhodamine. Long-term stability was observed over several months in both aqueous and organic phases. The release of the dye from the capsules could be triggered using laccase. Consequently, such enzyme-responsive nanocarriers were proposed for agricultural applications. The same group described later solid, porous, and core-shell microparticulate systems prepared from methacrylated kraft lignin by radical-initiated emulsion polymerization.[52] The morphology of the capsules depended on the proportion of dispersed hydrophobic phase in the emulsion. Time-dependent release of 2-propylpyridine as a model hydrophobic molecule was also studied from the microparticles. As with nanocapsules,[65] laccase activity increased the release rate of the encapsulated cargo, but resulting changes in the lignin structure were not reported.

Emulsion approach was also used in a system including methacrylated kraft lignin, fungicide pyraclostrobin, and hexadecane or plant oil, which were dispersed from chloroform into an aqueous solution containing a low concentration of sodium dodecyl sulfate (SDS), resulting in an oil-in-water emulsion.[64] Cross-linking of lignin was initiated by the addition of a diamine soluble in both phases, and the product was recovered by evaporating chloroform from the emulsion. Loading capacities from 4% to 15% were reported at EE exceeding 94%.

Tong *et al.* used sonication to create very stable nanocapsules based on chemically modified lignin, *i.e.*, allylated lignosulfonate and trimethylolpropane tris(3-mercaptopropionate) as cross-linker. Cross-linking was achieved by a radical polymerization process using azobisisobutyronitrile as initiator that was activated upon the sonication of the oil in water emulsion. Using coumarin-6 as water-insoluble hydrophobic cargo, the authors showed a pH-dependent release characteristic of the bespoken capsules.[53]

In 2014, Zou *et al.*, synthesized multicore capsules by oil-in-water Pickering emulsion polymerization using lignin, styrene, divinylbenzene, and hexadecane.[89] Later, Yi *et al*. loaded isophorone diisocyanate (IPDI) as a healing reagent in multilayer composite microcapsules based on LNP-stabilized oil-in-water (O/W) Pickering emulsion templates.[90] Size control of the microcapsules is conducted by varying the lignin content and the oil:water volume ratios of the Pickering emulsions. The capsules exhibited unique characteristics of, *i.e.*, 81 wt% core ratio, excellent thermal stability and high durability in aqueous solution-submersion, as well as >90% mass retention in solution-submersion and air exposure tests. One of the merits of this work is that the internal phase of the capsule was solely made of the active agents in contrast to many aforementioned approaches in which the oil core functions as a solvent phase.

Within the patent literature, several examples are found in which the use of lignins, most often lignosulfonates and alkali lignins, is claimed for the generation of micro- and nanocapsules for the controlled release and storage applications. Only a few works, however, list lignin-based capsules in more specific examples, and only these shall be discussed here. Wurm *et al.* patented lignin-based capsules from an oil in water emulsion. The oil contains the hydrophobic active, and capsule stability is realized by cross-linking events, including click chemistry.[64] Aiming for agricultural applications, the patent details as an example encapsulation and release of pyraclostrobin. Already as early as 1970, lignosulfonate was used together with gelatin to generate capsules by coacervation; capsules were shown to be pH-responsive, as one might expect.[91] In 1992, microcapsules based on various lignins and cross-linking agents or co-polymers were reported in which were encapsulated an insecticide, a hormone, or a fertilizer for protection against UV-light prior to application to vegetation.[92] Microcapsules are obtained by combining an emulsion of lignin-covered droplets with a 'hardening' solution, which fixes the droplets that contain up to 30% (w/w) of lignin. In 1996, lignosulfonate microcapsules were patented for the protective encapsulation of parathion, an organophosphate insecticide, in high bloom gelatine.[76] The patented invention made use of the elevated UV-protection achieved by the use of lignosulfonate or its azo-derivative.

4.2.2 Sunscreen actives and pharmaceuticals

Many recent studies have used lignin-based micro- and nanocapsules for sunscreen applications, relying on the natural UV absorbance of lignin and making use of synergistic effects between conventional, normally very hydrophobic sunscreen actives and lignin.[45–48,93] Among these works, Qiu *et al.* synthesized by ultrasonication lignin nanocapsules from enzymatic hydrolysis lignin that was washed with water several times before its use in encapsulating chemical sunscreens, namely avobenzone (UV-A blocker) and octinoxate (UV-B blocker).[48] The authors showed that these capsules, dispersed in suncreens, combine the previously observed beneficial synergistic effect[45] with a long lasting performance, thanks to the gradual release of the actives from the lignin capsules.

Likewise to entrapment of actives in lignin NPs, pH-Responsive lignin-based complex micelles in green solvents were realised by Li *et al.* using purified alkali lignin, which was cationized and then self-assembled into lignin-based complex micelles with sodium dodecyl benzenesulfonate (SDBS) in an ethanol/water mixture.[81] The complex micelles showed to be pH-sensitive. Encapsulation efficiently protected ibuprofen in simulated intestinal fluid, and pH-triggering caused a controllable release.

4.2. Adsorption of enzymes and other active substances

Adsorption is a common method for the immobilization of enzymes, and especially lipases have been immobilized in lignin-based materials (Table 4). Lipase B from *Candida antarctica* was adsorbed on conjugate material of oxidized kraft lignin and amino-derivatized chitin with an BET surface area of 194 m^2/g.[94] Another work adsorbed lipase from *Candida rugose* on composite beads prepared by co-precipitation of lignin and cellulose from the ionic liquid 1-ethyl-3-methylimidazolium acetate.[95] Compared to the hydrolytic activity of beads containing only cellulose, the supplementation of kraft lignin into the beads increased lipase activity by a factor of 2.6. A higher activity increment by a factor of 3.2 was reported with lipase from *Mucor javanicus* that was first adsorbed on cationic lignin nanospheres and subsequently

entrapped in calcium alginate beads at an EE% of 96%.[96] The same authors showed that, in addition to lipases, immobilized cutinase from *Humicola insolens* catalyzes synthesis of butyl butyrate in a biphasic solvent mixture, with volume fractions of 90% water and 10% hexane.

Table 4. Lignin-based materials used for the adsorption of actives.

Carrier material	Active ingredient	EE (%)[a]	LC (wt%)[b]	Ref.
Cellulose/alkali lignin hydrogel beads	lipase	52	1.4	[95]
Chitin-KL composite	lipase	1.0–2.0	33–11	[97]
Silica-lignin composite (alkali lignin) [c]	lipase	1.7	42	[94]
Cationic LNPs (SKL)	lipase, cutinase	96	5.5	[96]
Acetic acid lignin from bamboo shoot shells	α-amylase	n.a.	1.9	[98]
Lignin NPs (OSL)	tyrosinase	71–90	12–15	[80]
Lignin nanotubes (various lignins)	Plasmid DNA	n.a.	n.a.	[99]
Sugarcane bagasse soda lignin	methotrexate	n.a.	n.a.	[73]
Lignin microspheres (EHL)	λ-cyhalothrin	n.a.	5.7	[54]
Lignin-alginate hydrogel beads (hydrolytic lignin, Aldrich)	isoproturon	~40%[d]	0.002–0.005[e]	[100]
Aspen rot wood lignin	metamitron, metribuzin	n.a.	n.a.	[101]
Lignosulfonate-coated microcapsules (SLS)	picloram	50–88	93–97	[66]

[a] Entrapment efficiency. [b] Loading capacity in lignin carrier. [c] Alkali lignin from Sigma-Aldrich. [d] Calculated from the adsorption data. [e] Relative tot he moist lignin-alginate beads. KL, kraft lignin. SKL, softwood kraft lignin. OSL, organosolv lignin. EHL, enzymatic hydrolysis lignin. SLS, sodium lignosulfonate.

The activation of lipases occurs due to the amphiphilic properties of lignins[38,96,102] likewise to those of synthetic surfactants.[103–105] Lipase from *Aspergillus niger* was adsorbed on composite material consisting of chitin and oxidized kraft lignin.[97] The immobilization increased thermal and pH stability compared to the values of the native lipase. Besides lipases and cutinases, immobilization of porcine pancreatic α-amylase was reported by adsorption on acetic acid lignin isolated from bamboo shoot shells.[98] The immobilization efficiency was not determined, but the weight fraction of enzyme adsorbed on lignin was 1.9%. Activation of the enzyme likewise to that reported for the microbial lipases, but with unknown mechanism, was observed.

Lignins have also been used as precursors for the fabrication of nanotubes for affinity binding[56] and adsorption applications.[99] Ten *et al.* synthesized lignin nanotubes (LNTs) on a sacrificial alumina membrane template.[99] LNTs were used to transfer DNA into HeLa cells, and appeared better tolerated than carbon nanotubes (CNTs). Wahba *et al.* adsorbed methotrexate on sugarcane bagasse soda lignin for the treatment of rats induced with rheumatoid arthritis.[73] The release rate of this zwitterionic aromatic active ingredient was slower from lignin than from mesoporous silica or magnetite-silica nanocomposite, which was attributed to non-covalent attraction forces and the smaller average pore radius of lignin (4.9 nm) compared to the other adsorbents.

Due to the low cost and abundant availability, many lignins, including various crude lignin preparations, have been tested as adsorbents to carry pesticides for agricultural applications.[54,66,100,101] The UV-protective properties of lignin were utilized to increase the stability against UV-light of crystalline picloram, a systemic herbicide. LbL-coating of the crystals with a polymeric shell of alternating chitosan and sodium lignosulfonate layers additionally contributed towards its slow release in dilute aqueous ethanol solution.[66]

5. Covalent and non-covalent functionalization

Modifications and grafting of biological functionality generally aim at improving targeting and controlled release properties of lignin-based carriers. Compared to the abundant literature on the preparation of lignin-based nano- and microstructures as such, reports regarding their surface modification is relatively scarce. Caicedo *et al.* functionalized lignin nanotubes (LNTs) with avidin for their specific immobilization onto desthiobiotin-grafted glass surfaces.[56] They also demonstrated binding of LNTs carrying anti-concanavalin A onto glass functionalized with concanavalin A. Figueiredo *et al.* succinylated softwood kraft lignin and used the product to generate carboxylated lignin particles (CLNPs) that were further modified by grafting on them a block copolymer made of PEG, poly(histidine) and a cell-penetrating peptide.[63] The functionalized CLNPs showed spherical shape, cationic net charge, good stability in physiological media, and low cytotoxicity in all the tested cell lines. Benzazulene, a poorly water-soluble cytotoxic agent, was successfully loaded into the CLNPs, leading to a pH-sensitive release-profile and an enhanced antiproliferative effect in the different cancer cells compared with a normal endothelial cell line.

In addition to covalent grafting reactions, anionic LNPs have been modified by adsorption of cationic polymers such as poly(diallyldimethylammonium chloride), PDADMAC on their surfaces.[37,106] These monolayer-coated particles were stable even under strongly alkaline pH, and are thus usable in an extended range of applications. To reduce the carbon footprint of the product, Sipponen *et al.* adsorbed water-soluble cationized softwood kraft lignin on colloidal lignin particles, achieving a pH-dependent cationic net charge.[38] Such cationic lignin particles were used in emulsion stabilization[38] and enzyme immobilization,[96] but their cytocompatibility has not been assessed. Cationic polymers carrying quaternary amine headgroups are well known for their antimicrobial properties,[107,108] but cationic lignin particles have not been systematically studied for their possible cytoxicity for mammalian cells. Instead of cationic polymers, LNPs coated with proteins[109]

are expected to improve biocompatibility and reduce clearance rate; however, these important aspects are yet to be demonstrated under physiological conditions.

Silver ions and silver NPs have been used to incorporate antibacterial functionality into lignin materials. In 2015, Zhong *et al.* reported antibacterial silver nanoparticle composites in a matrix of poly(vinyl alcohol) and lignin isolated from spent pulping liquor.[110] Klapiszewski *et al.* prepared silica/lignin hybrid particles grafted with silver NPs.[111] Commercial silica material was modified with 2-aminoethyl-3-aminopropyltrimethoxysilane to increase affinity to kraft lignin oxidised with sodium periodate. Silver NPs grafted onto the resulting silica/lignin hybrids were stable and active against *Pcrossseudomonas aeruginosa*. Still in 2015, Richter *et al.* synthesized silver-ion-infused lignin nanoparticles as substitute of silver nanoparticles in antimicrobial applications. In contrast to the silver NPs, the silver-infused lignin particles may have a lower eco-toxicity due to a reduced durability of lignin NPs.[112]

6. Effects of nano- and microsized lignins on living organisms

The antibacterial activity and cytotoxicity of carbon nanomaterials depends *e.g.*, on the particle size, shape, surface chemistry, charge, and the type of cells incubated with them.[113,114] This interdependence has made it difficult to generalize the various mechanisms of the cytotoxic effects. Still, the phenomena have been studied quite extensively with silica NPs,[115–117] silver NPs,[118–121] fullerenes,[122] carbon nanotubes,[123,124] and carbon nanodots.[125] Lignin nanomaterials, being a relatively new research subject, have not yet been subjected to thorough safety assessment, but this should be done in the light of their recently demonstrated potential as drug and gene vectors and antimicrobial agents. Table 5 presents a comparison between nano- and microsized lignin along with inorganic and carbon nanomaterials and studies that have assessed their interactions with living organisms both *in vitro* and/or *in vivo*.

Table 5. Comparison of testing of lignin-, silica- and carbon-based nano- and microscaled materials on living organisms.

	Test type	Lignin-based materials	Inorganic NPs	Carbon-based nanomaterials
In vitro	Antiviral	No published studies	Silver NPs[126]	Functionalized fullerenes[127–129]
	Antibacterial	Silver-infused nanoparticles (SKL),[112] lignin-capped silver NPs (AL),[130,131] silica/lignin- silver NPs (KL),[111] acid-precipitated lignin particles (AL)[6]	Silver NPs,[132] Silica NPs (4 nm),[133] Nitric oxide-releasing silica NPs[134]	SWCNTs[135] Fullerene[136]
	Antifungal	Lignin-capped silver NPs (AL)[137]	Silver NPs,[138] Amphotericin B-conjugated silica NPs[139]	Functionalized MWCNTs[140]
	Anticancer	Lignin NPs with active substances (AL[a]),[62] Lignin-derived carbon dots (LS),[141] Emulsified *trans*-resveratrol (SLS)[142]	Silica NPs[143] Silica NPs loaded with active substances[144]	Fullerene[145] Crystalline Fullerene[146]
	Cell viability	Lignin microcapsules (KL),[51] LNPs (SKL),[60] Nanoemulsion encapsulating *trans*-resveratrol (SLS)[142]	Silica NPs,[147,148] Polyamidoamine dendrimer-capped silica NPs[149]	Carbon nanoparticles,[150] MWCNTs,[124] Crystalline Fullerene[122]
In vivo		Resveratrol-entrapped LNPs (AL[a]),[62] doxorubicin-entrapped LNPs,[61] methotrexate adsorbed on sugarcane bagasse soda lignin.[73]	Silica NPs,[115–117] silver NPs[118–121]	Carbon nanodots,[125] SWCNTs,[123] Crystalline Fullerene[122]

[a] Isolated from hydrothermally treated corn cobs. KL: kraft lignin; AL: alkali lignin; SLS: sodium lignosulfonate; SWCNTs: single-walled carbon nanotubes; MWCNTs: multi-walled carbon nanotubes. SLS: sodium lignosulfonate. LNPs. lignin nanoparticles.

Silver NPs[126] and fullerene derivatives[127–129] have been used for antiviral studies. In sharp contrast, no works on nano- or microscaled antiviral lignin materials are published, although antiviral properties have been reported for diverse type of lignins.[151–155] Silver NPs are very well-known for their antibacterial effects.[156,157] Recent works have also reported silica particles,[133,134] single-walled carbon nanotubes,[135] and fullerene[136] for antibacterial activity. The majority of antibacterial lignin materials take use of silver ions[112] or silver NPs[111,131,132,137] as active payload. Nevertheless, also metal-free acid-precipitated lignin particles were recently shown to possess antibacterial activity against Gram-negative plant pathogen strains.[6] The antimicrobial activity was related to the penetration of LNPs into the bacteria, under concomitant disruption of cellular functions. The authors did not study intracellular disintegration of LNPs, but leaching of low molecular weight lignin fragments is a plausible reason behind the antibacterial activity. Likewise to pristine silver NPs,[138] lignin-capped silver NPs[137] possessed antifungal activity.

The important findings from investigations of effects of lignin-based materials on living organisms are summarized in Table 6. Studies with fibroblast cells have shown that the commercially available alkali lignin is biocompatible both as such[131] and in form of alkali lignin-poly(3-hydroxybutyrate) composite nanofibers.[158] Moreover, the presence of residual lignin in cellulose hydrogel films increased cell viability compared to those observed on polystyrene or bleached cellulose hydrogel surfaces.[159] Evidence accumulated from studies that have used lignin NPs as carriers for anticancer drugs shows that the transformation into the nanoscale does not drastically alter biocompatibility of lignin *in vitro*. Lignin NPs are not antiproliferative towards cancerous cells in the absence of active payload, nor do they cause notable cytotoxic effects.[51,60,62,141] Endocytosis of coumarin 6 –loaded lignin microcapsules in Chinese hamster ovary cells showed no

interference of cell functions.[51] Although interactions of nanoscaled lignins with cells *in vitro* have attracted increasing attention in recent years,[6,51,60,62,112,137,160] there are very few *in vivo* studies on nano- and microscaled lignin materials. Dai *et al.* studied effects of resveratrol-loaded lignin NPs on cancer cells and red blood cells *in vitro* and on tumor-transplanted mice *in vivo*.[62] Non-loaded particles did not exhibit adverse haemolysis effects or reduction of cell viability, whereas resveratrol loaded particles showed significant inhibition of tumor growth *in vivo*.

Table 6. Findings from studies involving lignin-based nano- and microscaled materials *in vitro* and *in vivo*.

Type of lignin material	Type of organism(s)	Main findings	Reference
LMCs (AL)[a]	Chinese hamster ovary cells	Non-cytotoxic without encapsulated drug	[51]
Silver-infused LNPs (SKL)	*E. coli, P. aeruginosa, S. epidermidis, Ralstonia sp.*	Silver provides antibacterial effect, while antibacterial activity of empty particles was due to PDADMAC coating	[112]
Lignin-capped silver NPs (AL)[a]	*E. coli, S. aureus, A. niger*	Antifungal activity > antibacterial activity	[137]
Lignin-capped silver NPs and gold NPs (various lignins)	*E. coli, S. aureus*, 3T3 fibroblasts	Lignin-capped silver NPs and alkali lignin as such were non-cytotoxic to the fibroblasts	[131]
Resveratrol-loaded LNPs (lignin extracted from hydrothermally pretreated corn cobs with alkali)	Human lung cancer cells, murine Lewis lung carcinoma cells, mice	Lignin NPs were non-cytotoxic; entrapped resveratrol reduced tumor volume	[62]
LNPs (SKL)	Various cancerous and non-cancerous human cell-lines	No significant cytotoxic effects	[60]
Iron-containing LNPs (iron isopropoxide-modified SKL)	Various cancerous and non-cancerous human cell-lines	Some cytotoxic effects	[60]
Magnetite NP-loaded LNPs (SKL)	Various cancerous and non-cancerous human cell-lines	Some cytotoxic effects	[60]
LNPs (AL)[a]	NIH/3T3 fibroblast, mice implanted with B16F10 tumors	Some cytotoxic effects at high concentrations; LNPs from p-toluenesulfonic acid were more biocompatible than LNPs from THF:ethanol:water solvent system; histology did not show cytotoxicity of LNPs in mice, doxorubicin-loaded LNPs, but not bare LNPs, reduced tumor volume.	[61]
Acid-precipitated LNPs (AL)[a]	*P. syringae X. axonopodis, X. arboricola*	Antimicrobial activity	[6]
Soda lignin	Albino rats induced with rheumatoid arthritis	Intraperitoneal administration of methotrexate-loaded lignin alleviated symptoms and increased animal growth rates compared to control groups.	[73]
Lignin-PHB composite nanofibers (AL)[a]	NIH/3T3 fibroblast	No cytotoxicity, good biocompatibility	[158]
Lignin-PLA/PLLA nanofibers (AL)[b]	PC12, human mesenchymal stem cells, human dermal fibroblasts	Reduced ROS generation and cytotoxicity	[160]

[a] Alkali lignin from Sigma-Aldrich. [b] Alkali lignin from TCI. SKL, softwood kraft lignin,. LMCs, lignin microcapsules. LNPs, lignin nanoparticles. DMAEMA, 2-dimethylaminoethyl methacrylate; PDMAEMA, poly(2-Dimethylaminoethyl methacrylate).

A comparison of lignin to other organic nanomaterials reveals some intriguing differences. Hydroxylation of fullerene mitigates the generation of reactive oxygen species (ROS) that cause cytotoxicity of fullerene-based nanomaterials.[161] Although the number of available publications is still quite low, it can be speculated that various oxygen-containing functional groups make lignin generally more biocompatible than oxygen-depleted carbon materials, due to an increased hydrophilicity and number of sites at which the metabolic system can attack. Stemming from their intrinsic polyphenol functionality, lignin NPs have been found to alleviate oxidative stress exerted by PLA[160] and to provide beneficial radical scavenging activity in nanocomposites.[2] In nanomedicine, lignin NPs did not cause notable generation of ROS, while particles of ~200 nm diameter exhibited selective uptake by cancerous cells.[60] It is important to note that excessive covalent modification may have detrimental effects on biocompatibility of lignin materials. Liu *et al.* found that 2-(dimethylamino)ethyl (DMAEMA) grafted lignin copolymers with 40–65 nm hydrodynamic radii enabled plasmid DNA binding for gene delivery purposes. The *in vitro* cytotoxicity of the copolymers increased with increasing copolymer length; however, 5–6 DMAEMA units enabled transfection with sufficient biocompatibility.

7. Technical challenges

Nano- and microscale lignin particles and capsules exhibit several promising features as carriers of bioactive molecules. The facts that lignin is a natural antioxidative compound, possess antimicrobial activity, and that lignin nanoparticles are, so far, considered non-toxic encourage work in this area. However, there are also challenges related to the solvents used in the fabrication process that may restrain applicability of the formed lignin nanomaterials. Especially medically administered materials are very strictly regulated, and traces of harmful solvents will not be accepted. To this end, the use of safe and green solvents is preferable. However, there should not be major setbacks in yield of performance of materials fabricated from biocompatible solvents. Recently, aqueous acetone has been used in the preparation of LNPs at a mass yield of 88%.[2] Li *et al.* described precipitation of ethanol solutions of kraft lignin by the addition of water induces formation of nanocapsule structures.[43] However, in that case only a fraction of kraft lignin was soluble in ethanol and could be used in the process. Sipponen *et al.* showed that aqueous ethanol solubilized 87% of wheat straw soda lignin that can be

used for the entrapment actives such as budesonide in LNPs.[1] The drawback of aqueous ethanol solvent mixture is that the concentration of colloidally stable LNPs that can be prepared by direct non-solvent precipitation is lower than that achieved with THF or acetone. New solvent systems such as aqueous p-toluenesulfonic acid hold potential to increase concentration of LNPs,[61] but more work is needed to assess their performance and overall production cost. Overall, there is an obvious need for engineered nano- and micromaterial processes that minimize use of harmful solvents without compromising the dynamic range of nano- and microcarrier fabrication.

Another type of technical challenge arises from the structural features of lignin as the starting material itself in connection with the regulatory issues discussed in Section 8. Regardless of the type of lignin chosen for the application, it usually comes in form of a structurally and physico-chemically heterogeneous mixture of substances.[19,162] Improved understanding of the starting materials and their reactivity is a prerequisite to control the cytotoxicity and eco-toxicity profiles of lignin-based micro- and nanostructure. The use of fractionated lignins will here only reduce the problem, but not fully resolve it. A more promising route might be the genetic engineering of plants in a way that they produce a 'more uniform' lignin, which then can be isolated without drastic structural changes.[163,164] This, however, causes different regulatory and legal issues in other, related fields.

8. Regulatory and toxicological aspects

While composed of materials that are well known since decades, evaluation of engineered nanomaterials poses a particular regulatory challenge.[165,166] The European Commission (EC) and the Food and Drug Administration (FDA) are directing efforts towards more unified testing and assessment criteria.[167–169] While in the past membership to the family of nanomaterials was granted on the basis of physical dimensions, modern regulation and guidelines by the FDA and the EC are going away from this strict size-dependence, and extend the family also to micro-sized agglomerates. Recent research developments have pursued standardized toxicological assessment of nanomaterials,[166,170–172] and proposed undertaking several prerequisite steps during the nanoparticle generation and optimization stages to improve the eco-toxicity analysis of nanomaterials.[173] This is all the more noteworthy, since nanomaterials are penetrating all sectors of modern life: biomedical materials, home and personal care, agricultural products, etc. Moreover, engineered nanomaterials, also those composed of otherwise innocuous natural substances, pose yet not fully understood short- and long-term effects.

Being a natural substance, basic eco-toxicity of lignin is not a problem as such; problems are more likely to arise from chemical alteration during or for the fabrication process. Materials in which the nanostructure is due only to macromolecular interactions of lignin pose intrinsically less problems compared to those containing chemically modified lignins. For example, cross-linking of lignin can drastically reduce biodegradability by blocking the via the phenolic OH-groups, which are primary site of attack within the enzymatic degradation of lignins in nature. One problem remains, however, independent of whether a lignin was modified or not: a micro- or nanoparticle represents always an augmented local concentration in compact form in the environment that as such is not natural. This fact can lead to an 'unexpected' eco-toxicity such as increased occurrence of Fenton-like oxidation products.[174] It is obvious that more research is needed to shed light on the nanosafety and environmental impacts of lignin-based nanomaterials in various emerging applications.

9. Summary and Outlook

Utilization of lignin in materials science yet has mainly exploited its "passive" functionality, *i.e.*, adsorption and entrapment of actives, antioxidant, antimicrobial, UV-scavenging activity, pH-triggered release, etc. We are now approaching the watershed at which efforts will be focused on the design of lignin-based "active" functional materials. Biopolymer engineering with lignin will likely take inspiration from advanced materials developed from other polymers and colloidal materials. There is also a profound strive to extend the toolbox of different well-defined nanoscale building blocks. We envision that standardization of the production of spherical lignin particles, lignin capsules, and lignin nanofibers will lead to consistent material properties and foster the development of the next-generation materials. To facilitate assessment of various lignin-based carrier materials, we urge the researchers to adopt unambiguous reporting of encapsulation efficiency and loading capacity values along other meaningful results.

Many of the reports focus on finding use for lignin but not so much about taking advantage of the specific properties of lignin. The future is to more actively utilize the special chemistry of lignin such as pH-responsive solubility and tailorable thermal properties for added value. Specific functionality is important for many applications in biomedicine, biosensors, isomeric purification, and other high value-added applications. Materials carrying more than one active substance,[175] with synergistic simultaneous action or potential for controlled sequential release will be the next generation systems. Typical controlled release materials are composed of an enzyme-sensitive substrate linked to another component that leads to changes in macroscopic conformation.[176] Many of the systems developed thus far involve polymer hydrogels, including nanoparticle-polymer hydrogels,[175] which can also be formed from lignin.[2,96]

In addition to the first exploratory activities here reviewed, it is imperative, in order to develop efficient and sustainable new soft materials, to focus on clear-cut approaches using green chemistry techniques. More specifically, the possible fields of applications of active delivery require the use of non-toxic solvents that would possibly remain in traces in the final product. In addition, for the same issues of human toxicity or

environmental hazard, the new technologies should clearly avoid use of covalent cross-linking agents.

Advanced lignin-based materials will consists of engineered signaling chains that lead to the synthesis of the active form of the active only upon response of specific external stimuli. Such systems are already established in polymer science, and typically involve changes in solubility or macromolecular conformation.[177] The stimuli can be based on changes in thermal, light, pH, electrolyte concentration, properties of the external fluid, moisture, binding of a ligand, mechanical forces, electric, magnetic, and sonic fields, etc. For instance, ultrasonic treatment is known to assemble lignin particles into shells of microcapsule in emulsions.[51] Targeted and non-excessive dosing of active substances is an objective in medicinal applications because of the systemic toxicity and harmful side effects of many drugs. In addition to nanomedicine, a prominent large-scale application potential exists in agricultural and industrial applications such as stimuli-responsive release of plant protection agents, sensory/protective environmental materials, and immobilization of enzymes or cells for processing and upgrading of organic substances. Development of standardized purification procedures and demonstration of safety of various lignin grades remain of outmost importance for future applications in food, nanomedicine and healthcare materials.

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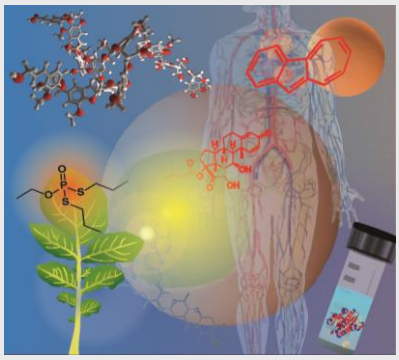
Entry for the Table of Contents

REVIEW

Lignin is an emerging natural substance for carrier systems with applications in biomedicine, agricultural chemistry, and biocatalysis. Sipponen *et al.* review current trends and challenges on the way to novel applications.



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Lignin for nano- and microscaled carrier systems: applications, trends and challenges