

This article was downloaded by: [Universita CA Foscari]

On: 14 November 2012, At: 05:29

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office:
Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Environmental Technology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tent20>

A New Procedure for the Monitoring of Cationic Detergents in Solution

M. Bragadin, P. Dell'Antone, G. Perin, S. Manente, A. Iero & G. Scutari
Version of record first published: 11 May 2010.

To cite this article: M. Bragadin, P. Dell'Antone, G. Perin, S. Manente, A. Iero & G. Scutari (2000): A New Procedure for the Monitoring of Cationic Detergents in Solution, *Environmental Technology*, 21:8, 937-939

To link to this article: <http://dx.doi.org/10.1080/09593332108618056>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

A NEW PROCEDURE FOR THE MONITORING OF CATIONIC DETERGENTS IN SOLUTION

M. BRAGADIN^{1*}, P. DELL'ANTONE², G. PERIN¹, S. MANENTE¹, A. IERO¹ AND G. SCUTARI³

¹ Dipartimento di Scienze Ambientali, Università di Venezia, DD 2137 30123 Venezia, Italy

² Dipartimento di Scienze Biomediche Sperimentali, Università di Padova, Via Trieste 75 35121 Padova, Italy

³ Dipartimento di Chimica Biologica, Università di Padova, Viale G. Colombo 3 35121 Padova, Italy

(Received 8 November 1999; Accepted 10 March 2000)

ABSTRACT

The paper describes a new procedure for the selective monitoring of cationic surfactants in solution. The procedure is based on the fact that cationic surfactants are accumulated inside mitochondria by a potential-driven mechanism. Once inside, the surfactant induces the release of the dye Safranin, previously accumulated inside mitochondria. Therefore the monitoring consists of a direct spectrophotometric measure of the rate of release of safranin in the resuspending medium containing the cationic surfactant.

Keywords: Cationic surfactants, mitochondria, safranin

INTRODUCTION

Surfactants are chemical compounds of great environmental interest. Anionic and nonionic surfactants are generally employed as detergents. However, the use of cationic detergents, usually employed as softeners, is increasing more and more [1]. The extensive use of detergents in industries and in households utilizations leads to unavoidable pollution problems from these compounds in natural waters. As a consequence, analytical procedures for their detection are required.

The most abundant cationic detergents are in the form of trimethyl aliphatic-chain ammonium salts in which the aliphatic chain ranges between 12 and 16 carbon atoms. It has been demonstrated that such compounds enter the mitochondria [2] and that the negative potential at the inner surface of the mitochondrial membrane is responsible for their uptake. This mechanism prompted us to investigate on analytical procedures based on a mitochondrial biosensor for the selective monitoring of cationic surfactants. The method is based on the release of the dye safranin (S), previously accumulated within energised mitochondria, when mitochondria uptakes the cationic surfactant. Safranin being a dye, can be monitored by spectrophotometry.

As a model for surfactants uptake cetyltrimethyl ammonium $\text{CH}_3\text{-(CH}_2\text{)}_{15}\text{-N(CH}_3\text{)}_3^+$ (CTAB) has been employed.

MATERIALS AND METHODS

All reagents were of analytical grade. Safranin and cetyltrimethylammonium bromide (CTAB) were supplied by SIGMA (Milan).

Rat liver mitochondria were prepared following the usual procedures [3]. The protein concentration was determined by the Lowry method [4].

Spectrophotometric measurements were performed by a Jenway 6400 Spectrophotometer (cell length 1 cm) at room temperature. The procedure employed for the spectrophotometric measurements is the following. The blank solution was made by mixing $0.2 \text{ mg}\cdot\text{ml}^{-1}$ of mitochondria, $10 \mu\text{M}$ Safranin, 0.25 M sucrose, 10 mM Tris buffer pH 7.4, 2 mM sodium succinate, 10 mM sodium acetate in 2.5 ml of aqueous solution. To this blank solution, the cationic surfactant was added and the change of absorbance was monitored as a function of the CTAB concentration. The spectrophotometer was calibrated against the blank solution at 530 nm .

RESULTS AND DISCUSSION

Safranin is a cationic dye which is captured by energised mitochondria [5]. The uptake is due to the presence of a negative-inside potential in energised mitochondria. Safranin (S) is therefore an indicator of the membrane potential. The accumulation of the dye inside the

mitochondria gives rise to a spectral shift [5] leading to a decrease of absorbance at a fixed wavelength. The energisation was achieved by addition of 2 mM sodium succinate. The absorbtion quenching upon addition of S is shown in Figure 1. Once an absorbance steady state had been reached, acetate was added. This lead to a further decrease of absorbance. This is due to the fact that acetate collapses ΔpH and consequently the $\Delta\Phi$ (the negative-inside potential) is enhanced [2]. In the new steady state, $\Delta\Phi$ has the highest value of about -220 m.V. Under these conditions, known amounts of CTAB are added in the solution. As soon as CTAB enters and accumulates within the mitochondria Safranin is released since the surfactant causes a membrane potential leak with loss of all accumulated compounds (including Safranin). The detergent effect and the consequent rate of S release is higher as the CTAB concentration is increased as shown in Figure 1.

Figure 2 shows the initial rate of the release of S at various CTAB concentrations. The initial rate corresponds to the slope of the plot absorbance (A) of S versus time (per milligram of protein) when the cationic detergent is added.

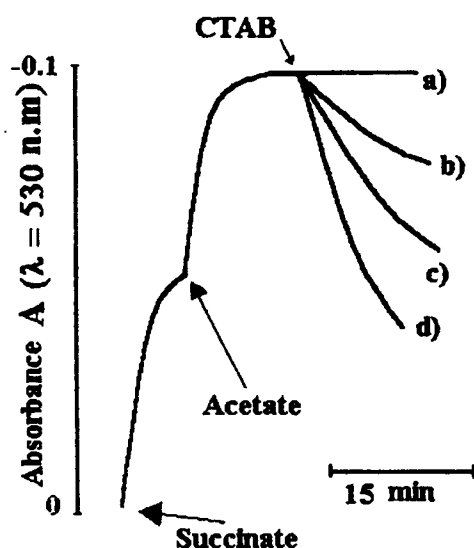


Figure 1. Typical experiment which shows the absorbance (at 530 nm) change during uptake and release of safranin in mitochondria. The medium (2.5 ml) contained: 0.25 M. sucrose, 20 mM Hepes pH 7.4, 2.5 mM $MgSO_4$, 0.5 mM EDTA, 10 μM safranin, 0.2 mg ml^{-1} of mitochondria and when indicated, after adjustment of the instrument at zero absorbance, 1 mM succinate, 10 mM acetate were added. The Safranin release is induced by increasing amounts of CTAB addition: (a) 1 μM , (b) 3 μM , (c) 4 μM , (d) 6 μM .

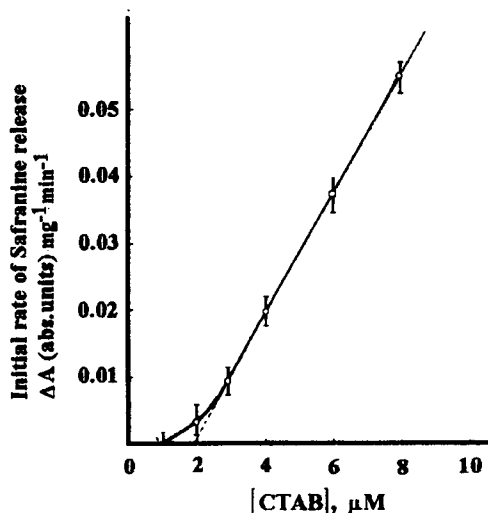


Figure 2. Initial rate of safranin efflux vs concentration of CTAB. Each point is the average from five replicates. Medium and conditions as in Figure 1.

The above plot is linear over the range 2-10 μM , the lower limit being roughly estimated by extrapolating the linear portion to $\Delta A \text{ mg}^{-1} \text{ min}^{-1} = 0$. The linear plot portion was obtained by linear regression analysis of experimental points taken as average over five independent measurements. Figure 2 shows also that at concentration of S below 2 μM the absorbance vs time is no longer linear.

This threshold is probably due to the fact that at a concentration of CTAB lower than 2 μM does not induce a membrane potential leak. The threshold value depends in fact on the ratio CTAB -mitochondrial protein and is higher at higher protein concentrations (not shown).

Interference effects were also examined. They could arise from the presence of chemical compounds such as phenols and organometallic compounds. Phenols interfere since these molecules enhance proton permeability and collapse $\Delta\Phi$ in a manner similar to that described for the surfactants. However, since phenols are complexed by albumin [6], their interference can be avoided by operating with an excess of albumin.

Organometallic compounds can interfere since they are also uncouplers of oxidative phosphorylation [7,8]. In this case the interference can be avoided by pretreating the sample at alkaline pH according to standard procedures reported in literature [9]. While the cationic detergents are not destroyed by this procedure, the organometallic compounds which are formed are no larger able to permeate through the mitochondrial membrane since they are not lipophilic cations.

Other interference could arise from compounds which inhibit the respiratory chain of mitochondria (i.e. cyanides). In this case the energisation of mitochondria and consequent potential formation can be performed by means of ATP, the results being the same.

REFERENCES

1. Fell B., Aktueller Stand-Absehbare Entwicklungen. *Tenside Surf. Det.*, **28**, 385-395 (1991).
2. Bragadin, M. and Dell'Antone, P., Mitochondrial bioenergetics as affected by cationic detergents. *Arch. Environ. Contamin. Toxicol.*, **39**, 280-284 (1996).
3. Pozzan, T, Bragadin, M. and Azzone, G.F., Disequilibrium between steady state Ca^{++} accumulation ratio and membrane potential. *Biochem.*, **16**, 5618-5625 (1977).
4. Lowry, O.H., Rosemberg, N.Y., Farr, A.L. and Randall, R.S., Protein measurement with folin phenol reagent. *J.Biol. Chem.* **193**, 265-275 (1951).
5. Colonna, R, Massari, S. and Azzone, G.F., The problem of cation-binding sites in the energized membrane of intact mitochondria. *Europ. J. Biochem.* **34**, 577-585 (1973).
6. Weinbach, E. and Garbus, J., The rapid restoration of respiratory control to uncoupled mitochondria. *J. Biol. Chem.* **25**, 3708-3713 (1996).
7. Bragadin, M. and Marton, D., A proposal for a new mechanism of interaction of trialkyltin (TAT) compounds with mitochondria. *J. Inorg. Biochem.* **68**, 75-78 (1997).
8. Bragadin, M., Marton, D., Iero, A., Manente, S., Perin, G., Rizzoli, V. and Scutari, G., a bioanalytical method for the monitoring of metal alkyls in solution. *Anal. Biochem.* **269**, 420-423 (1999).
9. Elschenbroich, C., Salzer, A., Organoelement compounds of the carbon group. In: *Organometallics A Concise Introduction*. VCH Weinheim (ed) New York Basel Cambridge pp121-137 (1991).