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On: 14 November 2012, At: 05:29
Publisher: Taylor & Francis
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Environmental Technology

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tent20

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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

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Environmental Technology, Vol. 21. pp 937-939
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A NEW PROCEDURE FOR THE MONITORING OF CATIONIC DETERGENTS IN SOLUTION

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(Received 8 November 1999; Aceepted 10 March 2000)

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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 Safranine, previously accumulated inside mitochondria. Therefore the monitoring consists of a direct spectrophotometric measure of the rate of release of safranine in the resuspending medium containing the cationic surfactant.

Keywords: Cationic surfactants, mitochondria, safranine

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 safranine (S), previously accumulated within energised mitochondria, when mitochondria uptakes the cationic surfactant. Safranine being a dye, can be monitored by spectrophotometry.

As a model for surfactants uptake cetyltrimethyl ammonium CH_3-$(CH_2)_{15}$-$N(CH_3)_3^+$ (CTAB) has been employed.

MATERIALS AND METHODS

All reagents were of analytical grade. Safranine 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 $mg \cdot ml^{-1}$ of mitochondria, 10 μM Safranine, 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

Safranine 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. Safranine (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 ΔΦ (the negative-inside potential) is enhanced [2]. In the new steady state, ΔΦ 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 Safranine is released since the surfactant causes a membrane potential leak with loss of all accumulated compounds (including Safranine). 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.

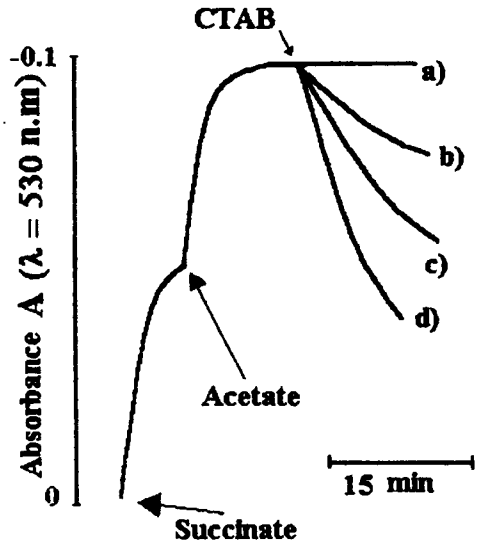

Figure 1. Typical experiment which shows the absorbance (at 530 nm) change during uptake and release of safranine in mitochondria. The medium (2.5 ml) contained: 0.25 M. sucrose, 20 mM Hepes pH 7.4, 2.5 mM $MgSO_4$ 0.5 mM EDTA, 10 μM safranine, 0.2 mg ml^{-1} of mitochomdria and when indicated, after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

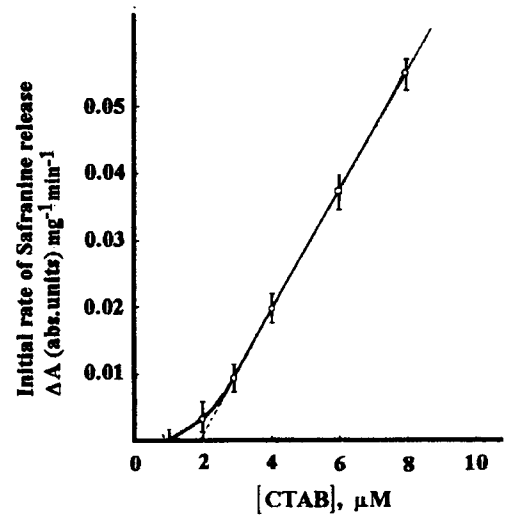

Figure 2. Initial rate of safranine efflux vs concentration of CTAB. Each point is the average from five replicates. Medium and conditions as in Figure 1.

The above plot is linear over the range 2-10 μM, the lower limit being roughly estimated by extrapolating the linear portion to ΔA mg^{-1} 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 ΔΦ in a manner similar to that described for the surfactants. However, since phenols are complexed by albimun [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.

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