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Dedicated to Professor Victor-Emanuel Sahini
on the occasion of his 80th anniversary

INTERACTION OF ACTINOMYCIN D WITH ANIONIC SURFACTANT, SODIUM DODECYL SULPHATE: SPECTRAL AND ELECTROCHEMICAL INVESTIGATIONS

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The interaction of actinomycin D with an anionic surfactant, sodium dodecyl sulphate (SDS) is investigated using spectral (UV-Vis absorption) and electrochemical (cyclic and linear voltammetry) methods. Both absorption and cyclic voltammetry results have outlined two distinct processes depending on the surfactant concentration. In the premicellar range the variation of the absorbance and of the peak current was assigned to the hydrophobic interaction of the drug with the surfactant molecules (Process I). At SDS concentrations higher than CMC a second type of interaction is observed, corresponding to the encapsulation of the drug into micelles, predominantly in monomeric form (Process II). Both processes were analyzed by nonlinear fitting using different interaction models; the binding constants and diffusion coefficients of the free and bound drug were determined.

INTRODUCTION

Conventional anticancer drugs have, besides their expected therapeutical effect, an undesirable cytotoxicity; indiscriminate destruction of tumor and normal cells limits their therapeutic dose and routes of administration. A way to overcome the toxic side effects of these drugs and to increase drug bioavailability is to change their pharmacological behavior by the incorporation or association with different drug carrier systems.[1,2] In this context, the utilization of surfactant micelles as drug carriers presents some advantages in comparison with other alternatives like soluble polymers and liposomes.[3] Micellar systems can solubilize poorly soluble drugs and thus increase their bioavailability, they can stay in the body (blood) long enough to provide gradual accumulation in the required area, and their sizes permit them to accumulate in areas with leaky vasculature.[4] In this context, numerous drug delivery and drug targeting systems have been studied in an attempt to minimize drug degradation and loss, to prevent harmful side effects and to improve drug bioavailability.

Actinomycin D is an anticancer antibiotic that contains a 2-aminophenoxazin-3-one chromophore and two cyclic pentapeptide lactones. This drug has been used clinically for the treatment of highly malignant tumors and also in combination with other anticancer agents to treat high-risk tumors,[5] their pharmacological action being attributed to the interaction with DNA *via* intercalation of the planar chromophore, preferable at the CpC sequence with the two pentapeptide rings resting on the minor groove.[6,7]

Similarly with the anthracycline antibiotics, the quinone - imine structure of actinomycin D may be a site for the bioreduction of this drug to reactive radical intermediates,[8] which can either cause alkylation of DNA and strand scission, or are capable to transfer the electron to molecular

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oxygen with formation of superoxide anion and hydroxyl radicals, responsible for cellular damages and cardiotoxicity.

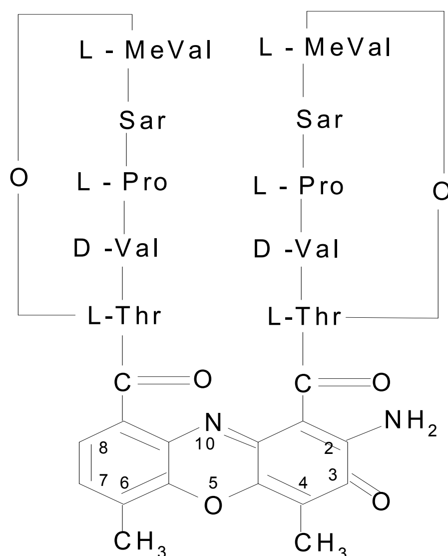

Fig. 1 - Molecular structure of actinomycin D.

The extent of interaction between the drugs and the surfactants can be best described by the hydrophobic effect (primarily determined by the hydrophobic surface area of the drug molecule) and the electrostatic effect (primarily determined by the charge associated with the drug molecule as well as the surfactant molecules).[9]

The aim of the present paper is to investigate the interaction of actinomycin D with an anionic surfactant, sodium dodecyl sulphate (SDS) in submicellar and micellar concentration range, using spectral (UV-Vis absorption) and electrochemical (cyclic and linear voltammetry) methods. If spectral methods are usually employed in the study of intermolecular interactions, in recent years electrochemical methods have gained growing interest in this type of studies due to their simplicity, rapidness and economy.[10-12]

EXPERIMENTAL

Actinomycin D and SDS were obtained from Sigma. Electrochemical experiments were performed in phosphate buffer (pH 7.4), at a VOLTALAB-32 electrochemical device, using a Pt-EDI 101 rotating disc working electrode of 2mm diameter, a Pt counter electrode and SCE reference electrode. The spectrophotometric measurements were carried out in a Unicam Helios-α spectrophotometer. Experiments were performed at room temperature in concentration range 1×10^{-5} – 5×10^{-3} M for spectral and respectively electrochemical experiments. Electrochemical parameters were evaluated using direct analysis followed by Digisim simulations with the BAS Digisim simulator 3.03.

RESULTS AND DISCUSSION

1. Spectral results

Absorption spectra of actinomycin D in the absence and in the presence of different SDS concentrations are presented in Fig. 2 a,b.

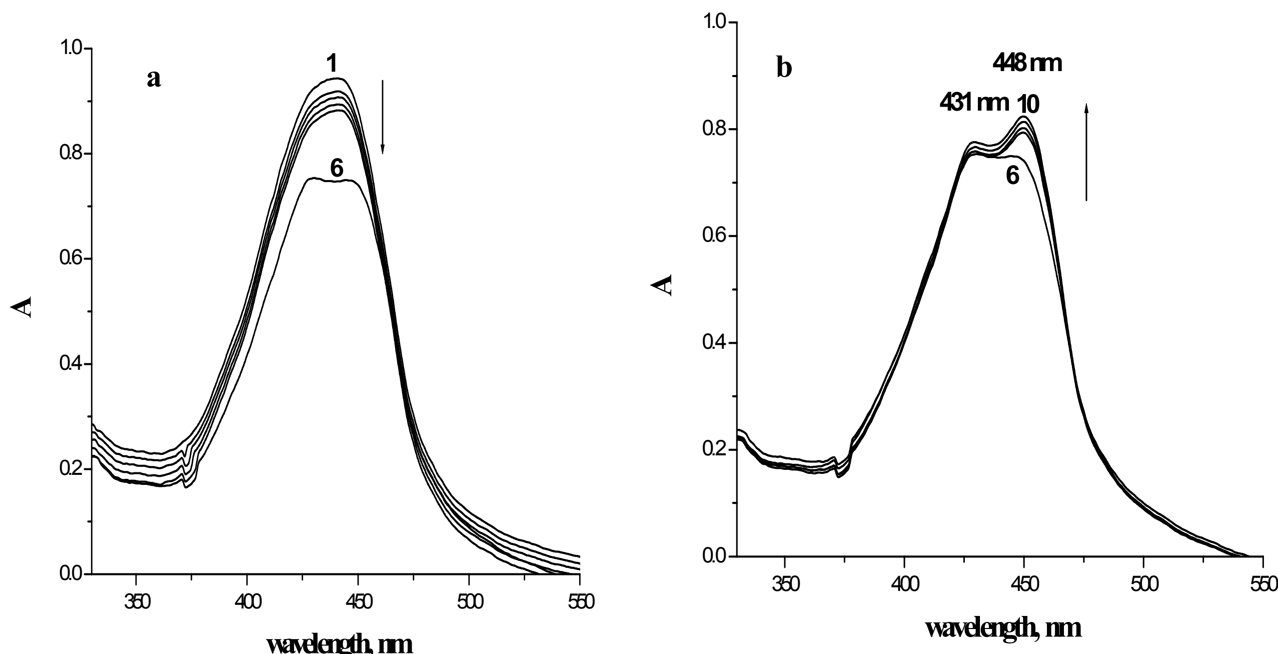

Fig. 2 – Absorption spectra of actinomycin D in the presence of different surfactant concentrations: (a) spectra 1 – 6, $C_{SDS} = 0 – 2.62 \times 10^{-3}$ M; (b) spectra 6 – 10, $C_{SDS} = 2.62 \times 10^{-3}$ M – 1.31×10^{-2} M.

It can be observed that in the absence of surfactant, the absorption spectra of actinomycin D present a broad maximum around 440 nm, consisting in two overlapping bands, assigned to the monomer (band at 448 nm) and respectively dimer (band at 431 nm). On gradual addition of SDS, a hypochromic effect is observed on both bands (Fig. 2a), up to a concentration of SDS of about $8x10^{-4}$ M. For higher SDS concentrations, the absorption maximum is splitted into two peaks at 431 nm and 448 nm and the absorbance increases (Fig. 2b).

The absorption variation as a function of surfactant concentration (Fig. 3) indicates that initially, as surfactant is added, the absorbance of actinomycin D declines significantly until a breaking point is reached (Process I). After a short range of SDS concentrations where it remains constant at the lower value, the absorbance increases again (Process II, Fig. 3). It may be noted that the flat portion of the binding curve is between $4\text{-}5x10^{-4}$ and $4x10^{-3}$ M, i.e. in the proximity of the critical micellar concentration of SDS in 0.1 M saline solutions, ($1.4x10^{-3}$ M).[13]

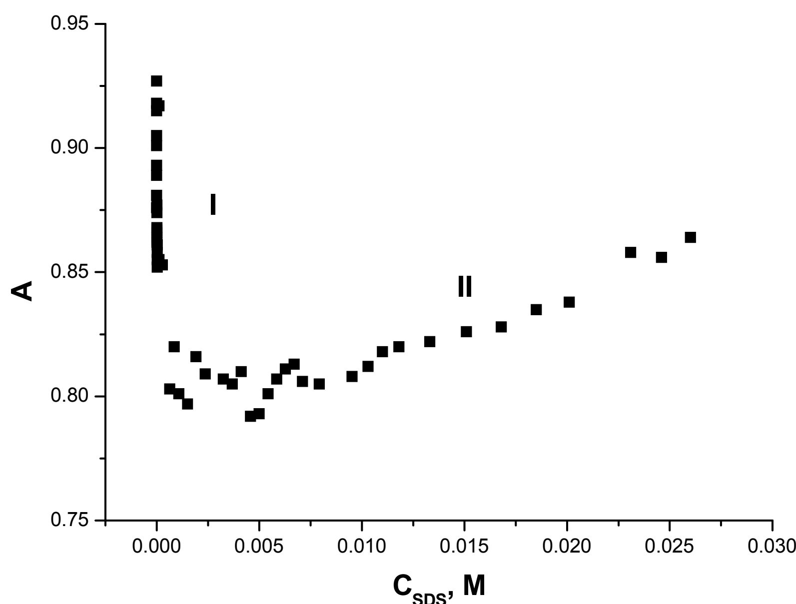

Fig. 3 – Binding curve of actinomycin D to SDS in phosphate buffer (pH 7.4) obtained from spectral data: Process I – premicellar range, Process II – micellar range.

Both processes were analyzed assuming a 1:1 interaction between the drug and SDS:

M + L = ML

The association constant is given by:

$$K = \frac{C_b}{(C_T - C_b)(C_{SDS} - C_b)} \quad (1)$$

where C_T is the total concentration of actinomycin D, C_b is the concentration of the bound actinomycin D and C_{SDS} is the total concentration of surfactant.

The absorbance of the solution at a wavelength in the band of actinomycin D, where the ligand is supposed not to absorb, is given by:

$$A = \varepsilon_f C_f + \varepsilon_b C_b \quad (2)$$

Taking into account that $C_T = C_b + C_f$, and replacing for C_b the expression resulting from equation (1), the following equation is obtained for the variation of the absorbance in function of the total SDS concentration:

$$\Delta A = 0.5(\varepsilon_f - \varepsilon_b)(C_T + C_{SDS} + 1/K) - \sqrt{(C_T + C_{SDS} + 1/K)^2 - 4C_T C_{SDS}} \quad (3)$$

where $\Delta A = A_0 - A$, ε_f, ε_b – the molar absorption coefficient of the free and bound actinomycin D.

If the concentration of the bound species is smaller than the ligand initial concentration, a simplified formula is obtained:

$$A = \frac{A_0 + A_{inf} K C_{SDS}}{1 + K C_{SDS}} \quad (4)$$

where A_0 and A_{inf} are the absorbances in the absence and in the presence of SDS.

Nonlinear fitting using equation (3) is presented in Fig. 4a. Three different experiments yield for Process I an average equilibrium constant of $(3.71 \pm 0.5)x10^3$ M^{-1} and ε_b = 19804 $M^{-1}cm^{-1}$. It can be observed that the binding constant is similar to the dimerization constant (K_d = $(3.65x10^3$ M^{-1}) of the drug in phosphate buffer solution,[14] and the molar absorption coefficient is between the value obtained for the monomer (ε = 24400 $M^{-1}cm^{-1}$)[15] and that of the dimer (ε = 11944 $M^{-1}cm^{-1}$).[14] These results seem to indicate that, as in the case of other dyes,[13] the dimerization of the drug is a non-negligible contribution to the actinomycin D - SDS interaction in the premicellar concentration range (Process I).

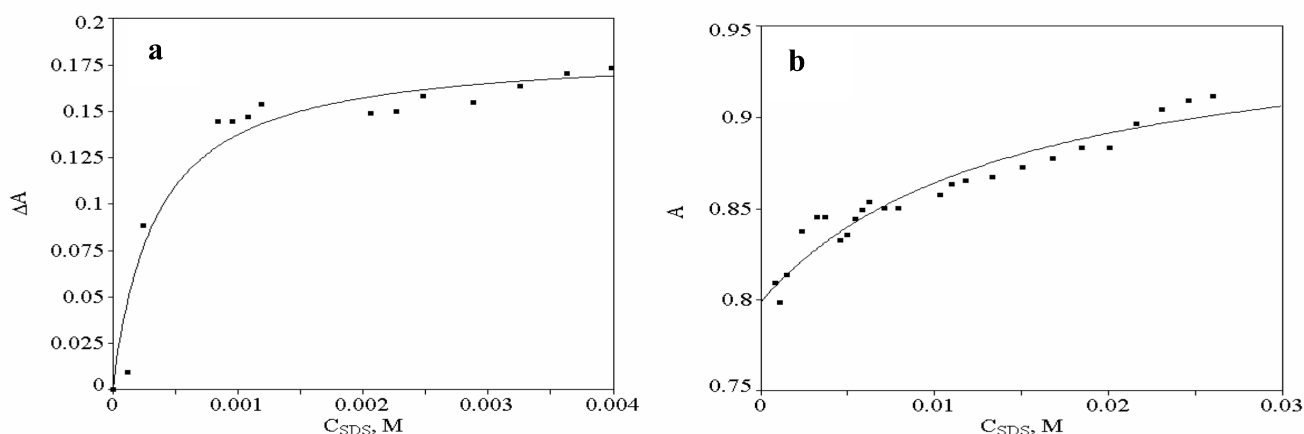

Fig. 4 – (a) Nonlinear fitting of the experimental spectral data corresponding to the Process I using equation (3); (b) Nonlinear fitting of the experimental spectral data corresponding to the Process II using equation (4). The binding parameters are given in the text.

The Process II, occurring at SDS concentrations higher than CMC, was analyzed by nonlinear regression assuming a 1:1 interaction between the drug and the SDS micelle, (Fig. 4b) using the equation (4).

The results obtained are: K = 71 M^{-1} and ε_b = 25158 $M^{-1}cm^{-1}$. This value of binding constant is in the range of the solubilization constants of other drugs and dyes in SDS micelles.[16,17] Also, it can be observed that the molar absorption coefficient presents similar values with the molar absorption coefficient of free monomer, attesting that the drug is incorporated in micelles as monomer.

2. Electrochemical results

The cyclic voltammograms of actinomycin D in phosphate buffer, in the potential range 500 ÷ -1500 mV, in the absence and in the presence of different SDS concentrations are presented in Fig. 5.

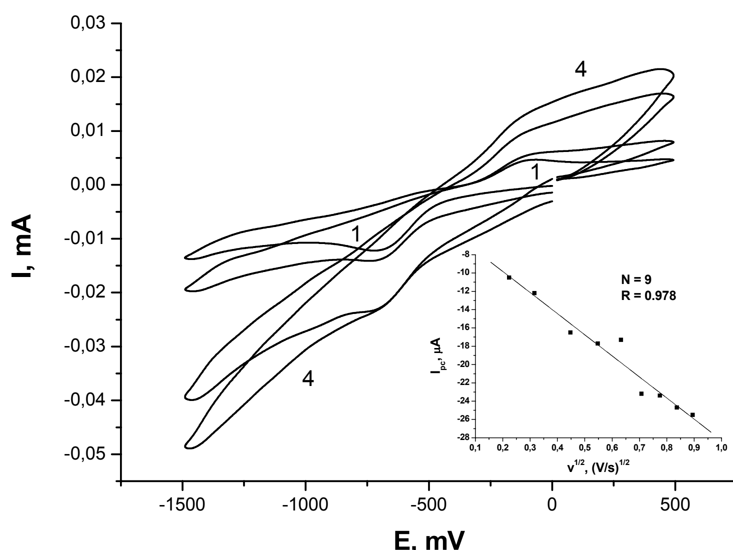

Fig. 5 – Cyclic voltammograms of actinomycin D (C=1×10^{-3} M) in phosphate buffer in the presence of different SDS concentrations: 1 – 0; 2 – 4.02×10^{-3} M; 3 – 9.33×10^{-3} M; 4 – 11.97×10^{-3} M (v = 0.1 V/s); inset – dependence of the cathodic peak current on $v^{1/2}$ in the absence of surfactant. From the slope $D_f = 0.7\times10^{-5}$ cm^2s^{-1} was obtained.

In the absence of SDS the first reduction couple was analyzed by cyclic and linear RDE voltammetry and was assigned to a slow monoelectronic reduction of the drug to the corresponding anion radical. The standard apparent ET rate determined by Digisim simulation was $2.3\text{x}10^{-5}$ cm s^{-1}. The diffusion coefficient of actinomycin D in the absence of surfactant, D_f, was determined from the slope of the plot of the peak current I_{pc} vs. $v^{1/2}$ (inset Fig. 5) and a value of $0.7\text{x}10^{-5}$ cm^2s^{-1} was obtained.

The gradual addition of surfactant to the actinomycin D solution shifted the cathodic peak towards positive potential values and the peak current presents a dependence on the surfactant concentration quite similar to that of absorbance (Fig. 6). The two processes, i.e. a stiff increase of the cathodic peak current up to around CMC, (premicellar range - Process I), followed by a slight decrease at higher surfactant concentrations, (micellar range - Process II), are clearly evidenced (Fig. 6).

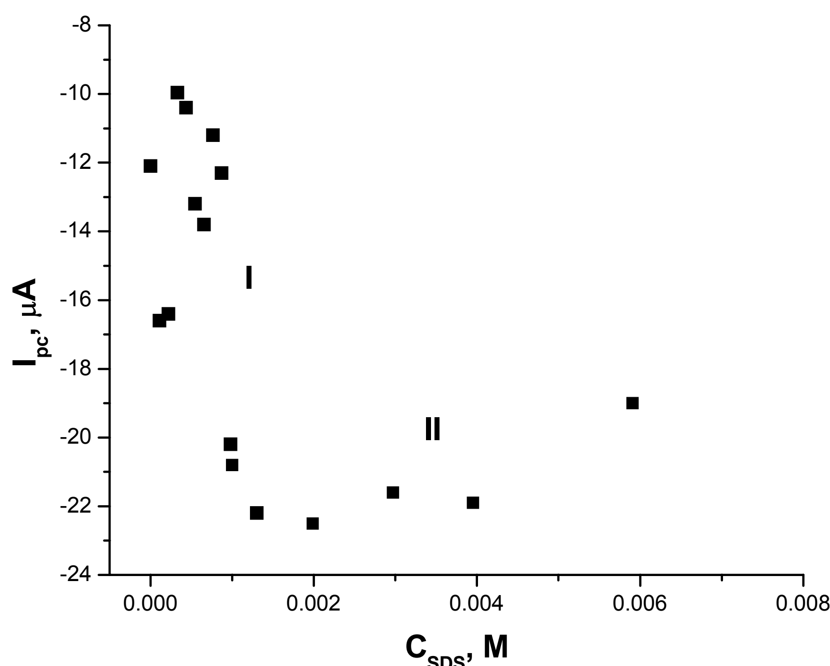

Fig. 6 – Cathodic peak current dependence on the surfactant (SDS) concentration: the two processes outlined by absorption spectra are also evidenced: Process I – premicellar range, Process II – micellar range.

Using cyclic voltammetry, information regarding the interaction may be obtained either from potential values or peak currents.

If the reduced form of the drug is stabilized by the interaction, the potential of the redox couple is shifted towards positive values and the interaction constant (K) and the number of ligands (p) may be determined using the following equation:[18]

$$E_{1/2}^{complex} - E_{1/2}^{0} = \frac{RT}{nF}\ln K + \frac{pRT}{nF}\ln C_{SDS} \quad (5)$$

The half-wave potential in the absence ($E_{1/2}^{0}$) and in the presence ($E_{1/2}^{complex}$) of different surfactant concentrations were evaluated from cyclic voltammetry as $(E_{pc} + E_{pa})/2$. From the plot of $\Delta E_{1/2}$ against the logarithm of the surfactant concentration, a binding constant, $K = 4.3\text{x}10^3$ M^{-1}, and p = 0.88 are obtained, attesting for a 1:1 drug - SDS complex.

The peak current for an irreversible electron transfer at 25°C is given by:

$$I = 2.99\text{x}10^5(\alpha n)^{1/2} A D_O^{1/2} C_O v^{1/2} \quad (6)$$

where n is the number of electrons transferred in the rate determining step, α the transfer coefficient, A the electrode surface and *v* is the sweep rate.

Assuming a 1:1 interaction between the drug and SDS, the total current at any SDS concentration is given by:

$$I = B[D_f^{1/2}C_f + D_b^{1/2}C_b] \quad (7)$$

With $C_T = C_b + C_f$, the expression of the peak current becomes:

$$I = B[D_f^{1/2}C_T - (D_f^{1/2} - D_b^{1/2})C_b] \quad (8)$$

where B represents all the constants ($2.99x10^5(\alpha n)^{1/2}A\nu^{1/2}$) and D_f and D_b are the diffusion coefficients of the free and bound drug, respectively. If I_0 stands for the current in the absence of ligand ($I_0 = BD_f^{1/2}C_T$) and I_{inf} for the current corresponding to a solution containing SDS in excess ($I_{inf} = BD_b^{1/2}C_b$), the current expression becomes:

$$I = \frac{I_0 + I_{inf}KC_{SDS}}{1 + KC_{SDS}} \quad (9)$$

A nonlinear regression fit of the Process I in the premicellar concentration range with equation (9), yielded the following results: $K = 4.5x10^3$ M^{-1} and I_{inf} = -22.82 μA, corresponding to $D_b = 3.0\times10^{-5}$ cm^2s^{-1} (Fig. 7), i.e. a binding constant close to that obtained from potential shift, and to the value determined from absorption spectra.

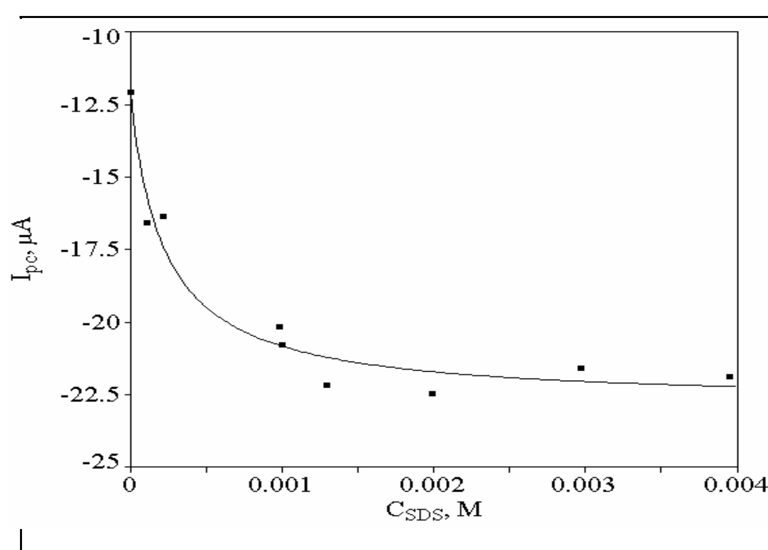

Fig. 7 – Nonlinear fitting of the experimental electrochemical data corresponding to the Process I using equation (9). The binding parameters are given in the text.

The value obtained for the diffusion coefficient of the drug - SDS aggregate in the proximity of CMC from peak current analysis, D_b, does not differ substantially from that of the free drug in aqueous solutions.

DISCUSSION

Both absorption and cyclic voltammetry results indicate two distinct interaction types, dependent on the surfactant concentration.

At very low concentration of surfactant a 1:1 binding, probably at specific sites, occurs. (Process I). For positively charged drugs this is predominantly electrostatic[17] whereas for proteins and not charged drugs like actinomycin D, it is predominantly hydrophobic. The aspect of the absorption variation in function of SDS concentration in the premicellar range is quite similar to the variation of the surface tension curve with SDS concentration in presence of myoglobin. In protein-surfactant systems, aggregation of the surfactant occurs at SDS concentrations of an order of magnitude smaller than CMC, and the phenomenon is known as protein-driven micellar aggregation.[19]

In the actinomycin D solution, increasing the SDS concentration (before CMC), formation of premicellar aggregates begins at a SDS concentration of about 5×10^{-4} M, i.e. micellar aggregation occurs at a concentration of surfactant which is much lower than in the absence of actinomycin D. The flat part of the curve in the following SDS concentration range (up to 4×10^{-3} M

in our case) corresponds to the cooperative binding to the actinomycin D-peptide sites, where a SDS molecule is already bound. This behaviour is supported by the analogy of the spectral results with those at the DNA-actinomycin D interaction at small and intermediate P/D ratios.[14] Actinomycin D, with two pentapeptide chains attached to a phenoxazone chromophore is generally considered as a model compound in the study of protein-DNA interactions. Both experimental and theoretical modeling data have outlined the major non-electrostatic nature to the binding free energy.[20-23]

The hydrophobic nature of the actinomycin D – SDS interaction is also supported by examination of the amino acids in the pentapeptide chains, all amino acids containing non-polar groups at neutral pH, and therefore hydrophobic interactions with the aliphatic chain of SDS are expected to prevail.

At SDS concentration higher than CMC, the absorption increases again, as result of the encapsulation of the drug into micelles, predominantly in monomeric form (Process II). Separate analysis of process II is justified, because at C_{SDS} > CMC the main process is drug-micelle interaction with a 1:1 binding stoichiometry, corresponding to the introduction of the monomeric drug into surfactant micelles.

The effect of ionic strength upon the drug – SDS interaction is somewhat similar to that of increasing SDS concentration: at low ionic strength (< 0.04 M) premicellar aggregates contain drug dimers and trimers, explaining the decrease of the monomer and dimer absorption bands (Process I), whereas at higher ionic strength (≥ 0.1 M), like the phosphate buffer used in our experiments (I = 0.15 M), monomers become predominant in micelles and the intensity of the monomer band increases (Process II). Higher surfactant concentration increases the solubility of the drug, while more salt will tend to produce larger micelles and sort out the drug from water into the micelle.[13]

CONCLUSIONS

The interaction of actinomycin D with an anionic surfactant was investigated using coupled cyclic and linear voltammetry with visible absorption spectroscopy. The results have outlined two distinct processes depending on the surfactant concentration. In the premicellar range the variation of the absorbance and peak current was assigned to the 1:1 interaction of the drug with molecular surfactant, mainly hydrophobic (Process I).

At SDS concentration higher than CMC, a second type of interaction is observed, correspondding to the encapsulation of the drug into micelles, predominantly in monomeric form (Process II). The transition domain between these two processes corresponds to the formation of premicellar aggregates, which occurs at a concentration of surfactant that is much lower than in the absence of actinomycin D and corresponds to the cooperative binding of the surfactant to the actinomycin D-peptide sites, where a SDS molecule is already bound. Nonlinear fitting of these processes based on spectral and electrochemical experimental data allows the quantitative characterization by binding constants and diffusion coefficients of the free and bound drug.

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