International Journal of Pharmaceutics 390 (2010) 100–106

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Coupled spectral and electrochemical evaluation of the anticancer drug mitoxantrone–sodium dodecyl sulfate interaction

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ARTICLE INFO

Article history:
Received 21 October 2009
Received in revised form
10 December 2009
Accepted 13 December 2009
Available online 26 February 2010

Keywords:
Mitoxantrone
SDS
Binding constant
Partition coefficient
Absorption spectroscopy
Cyclic voltammetry

ABSTRACT

In-vitro evaluation of the interaction of anticancer drug mitoxantrone with anionic surfactant sodium dodecyl sulfate (SDS), was performed in physiological conditions (phosphate buffer, pH 7.4) by spectral (UV–vis absorption) and electrochemical (cyclic and linear voltammetry) methods.

The stoichiometry of interaction, the binding constants and diffusion coefficients of free and bound drug were determined. The partition coefficient of mitoxantrone between aqueous phase and SDS micelles was calculated, and the results indicated that it is strongly dependent on the drug concentration. Both absorption and cyclic voltammetry results have outlined two distinct processes depending on the surfactant concentration: process I in premicellar range, assigned to the interaction of the drug with the surfactant molecules, when electrostatic forces play an important role in the formation of the mitoxantrone-SDS complex with a defined stoichiometry; and process II in micellar range, when the surfactant micelles are formed and the drug is encapsulated in micelles in monomer form. The spectral results have indicated that the drug is most probably located in the micelle surface layer, both electrostatic and polar interactions playing important role in the binding of drug to SDS micelles. Possible application of this system as a micellar drug–carrier was also considered.

1. Introduction

Physicochemical characterization of drug delivery systems, both in vitro and in vivo conditions is a research direction of major importance in last decades pharmaceutics, with high medical impact in the treatment of different diseases, including cancer (Schreier et al., 2000; Kostarelos, 2003). The clinical use of antitumor drugs is limited by their toxic side effects, especially cardiotoxicity (Minotti et al., 2004; Schimmel et al., 2004). The efficacy of treatment can be improved by the encapsulation of the drugs in different carrier systems, in order to ensure the transport to specific sites of action, to minimize drug degradation and loss, to prevent harmful side effects, and to increase drug bioavailability (Florence and Hussain, 2001; Rangel-Yagui et al., 2005). In this context, the utilization of micelles (colloidal-sized clusters formed by surfactants in solutions) as drug carriers provide some advantages in comparison with other alternatives like liposomes and polymers: micelles can be obtained in an easy and reproducible manner in large quantities and specific ligands can be attached to their outer surface in order to optimize the controlled releasing and specificity of pharmacological effect (Torchilin, 2001). Micelles offer a core/shell structure and, therefore, stay water-soluble, in comparison with polymeric carriers that may lead to precipitation in water (Jones and Leroux, 1999). By their size between 5 to 100 nm, micellar systems are intermediate between other drug carriers like antibodies, dextran and albumin (size smaller than 5 nm) and liposomes and microcapsules with size higher than 50 nm. Usual pharmaceutical micelles are between 10 and 80 nm and, therefore they can accumulate in areas with leaky vasculature (Torchilin, 2001; Rangel-Yagui et al., 2005). Also, surfactant micelles have been used as model systems for biomembranes to study the interactions of different compounds, including drug molecules, with biological membranes (Atta et al., 2007; Cudina et al., 2008). The nature and magnitude of drug-biological membranes interactions can determine the drug release from the carrier and the physico-chemical interactions of drugs with surfactant micelles can be understood as an approximation for their interactions with the biological surface. Drug interactions with heterogeneous media like micelles, vesicles or biomembranes induce changes in some physicochemical properties of drugs (solubility, spectroscopic and acid-base properties) (Chakraborty and Sarkar, 2005). A quantitative evaluation of the effect of micelles on the properties of pharmaceutical drugs requires the determination of the drug-micelle binding constant and the micelle-water partition coefficient, which are important parameters for the understanding of interactions with biological membranes, as well as for the quantitative structure-

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doi:10.1016/j.ijpharm.2009.12.064

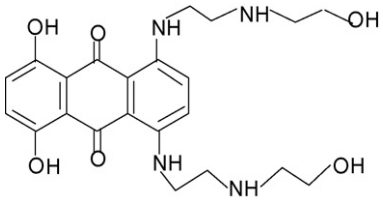

Fig. 1. Molecular structure of mitoxantrone.

reactivity relationship of drugs (Cudina et al., 2008). The extent of the drug–surfactant interaction, as well as analysis of the forces implied, can be described in terms of 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 and/or the surfactant molecules) (Khossravi, 1997).

If absorption spectroscopy is the current technique employed in this kind of studies, the use of electrochemical methods is much more recent (Zhao et al., 1999; Rauf et al., 2005). The use of spectral and electrochemical methods in conjunction, especially when charged species and/or electrostatic interactions are involved, presents the advantage to obtain complementary information: electrochemical methods give information regarding the stoichiometry of the interaction, the diffusion coefficients of the free and bound drug, whereas the spectral ones, more specific from a structural point of view, allow the determination of molar absorption coefficient of the different species involved. Mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)-amino]-ethyl]-amino]-9,10-anthracenedione) (Fig. 1) is an anthracenedione antitumor drug, active clinically in different types of cancer (Doughty et al., 2002; Nowoselac et al., 2004; Hagemeister et al., 2005), and developed to eliminate the side effects of anthracycline antibiotics, especially cardiotoxicity. The structure of mitoxantrone is shown in Fig. 1. It has a planar heterocyclic ring substituted with two positively charged nitrogen-containing side chains.

The aim of the present paper is to investigate the interaction of mitoxantrone with an anionic surfactant, sodium dodecyl sulfate, by coupling spectral (UV–vis absorption spectroscopy) and electrochemical (cyclic and linear voltammetry with stationary and rotating disc electrode, RDE) methods, in order to evidence the drug species involved, and to elucidate the nature of the interactions in premicellar and micellar range of concentrations, as a preliminary step in view of a possible application of this system as a drug carrier.

2. Materials and methods

Mitoxantrone hydrochloride and sodium dodecyl sulfate (SDS) were purchased from Sigma and used without further purification. Mitoxantrone concentration in phosphate buffer solution was determined spectrophotometrically at 660 nm, using the molar absorption coefficient $\varepsilon = 19{,}500\ M^{-1}\ cm^{-1}$ (Rosenberg et al., 1986). Experiments were performed at room temperature and double distilled water was used for the preparation of solutions. Electrochemical experiments were performed in phosphate buffer (pH 7.4, ionic strength 0.15 M), at a VOLTALAB-40 electrochemical device, using a cell equipped with three electrodes: Pt-EDI 101 rotating disc working electrode of 2 mm diameter, a Pt counter electrode and SCE reference electrode. The spectrophotometric measurements were carried out in a Unicam Helios-α spectrophotometer. Mitoxantrone–SDS (SDS micelles) binding constant and micelle/water partition coefficient were determined from the absorbances at $\lambda = 660$ nm of series of solutions containing a fixed drug concentration and increasing SDS concentrations, absorption measurements being made after 1–2 min, time sufficient to ensure the attainment of equilibrium. Even if the ligand (SDS) does not absorb at the analytical wavelength, the surfactant solution was added also to the reference cell so that the sample and the reference would have the same refraction index value. The spectral and electrochemical results are the average of 3–5 different experiments. The value of the critical micellar concentration (CMC) for SDS in presence of mitoxantrone was determined from the change in the absorption spectrum of mitoxantrone as the SDS concentration corresponding to the first point marking the increase of absorbance, which indicates the beginning of micelle formation (Samsonoff et al., 1986; Patist et al., 2000; Gokturk and Tuncay, 2003). Linear and non-linear fitting of experimental data was performed using Origin 7.0 software.

3. Results and discussion

3.1. Spectral results

Interaction of mitoxantrone with anionic surfactant SDS has been studied by UV–vis absorption spectroscopy in premicellar and micellar range concentration. In the absence of surfactant, the absorption spectra of mitoxantrone in phosphate buffer present two absorption maxima at 610 and 660 nm, corresponding, according to literature data (Lee and Dutta, 1989), to the dimer (D) and respectively, monomer (M) of the drug.

Fig. 2 shows the effect of different concentrations of SDS surfactant on the absorption spectrum of mitoxantrone. On gradual addition of SDS, a hypochromic effect is observed on both bands

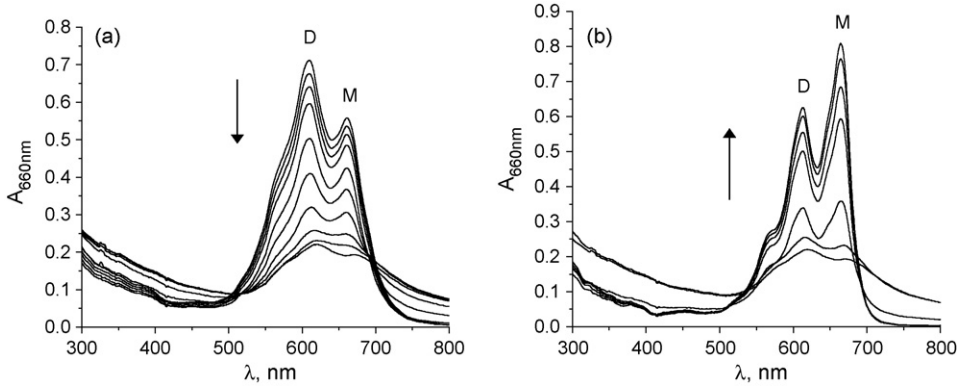

Fig. 2. Absorption spectra of mitoxantrone (2.86×10^{-5} M) in phosphate buffer in the presence of different SDS concentrations: (a) $C_{SDS} = 0$–7.28×10^{-4} M; (b) $C_{SDS} = 7.28 \times 10^{-4}$–$8.64 \times 10^{-3}$ M (the arrows indicate the decrease (a) and increase (b) of absorbance of the monomer and dimer bands).

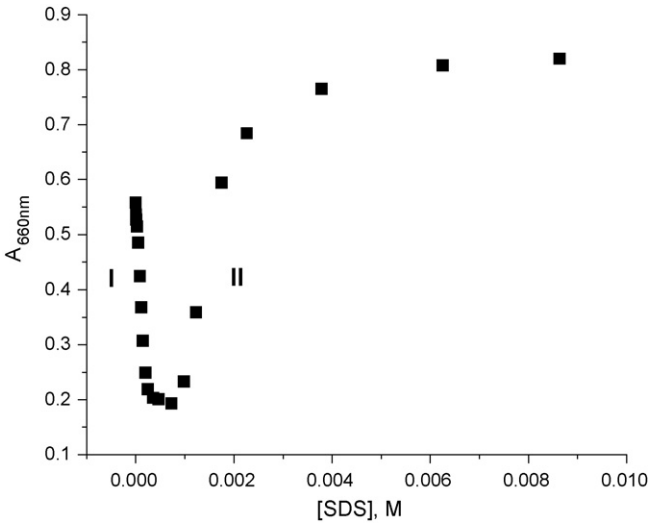

Fig. 3. Variation of the absorbance at 660 nm with SDS concentration (processes I and II are indicated).

with no shift in the absorbance maxima (Fig. 2a), up to a concentration of SDS of about 7.28×10^{-4} M. In neutral aqueous solution, mitoxantrone hydrochloride dissociates as anion (Cl^-) and bivalent cation due to the two protonated nitrogen atoms from lateral chains. In the same conditions, anionic surfactant SDS can exist as univalent anion monomers, oligomers or micelles. At SDS concentration smaller than CMC, the intensity of the absorption bands at 610 and 660 nm decrease (Figs. 2a and 3) and the drug-SDS complexes are formed due to the electrostatic interaction and hydrophobic forces, the reaction mainly taking place at the protonated nitrogen atoms from lateral chains. The two isobestic points in Fig. 2a sustain the idea of a specific interaction with a defined stoichiometry. As was indicated by Job's method of continuous variation and molar ratio method (Liu et al., 2007), the stoichiometry of mitoxantrone:SDS ion association complex is most probably 1:2.

The variation of absorbance at 660 nm as a function of surfactant concentration (Fig. 3) indicates two distinct processes: process I in premicellar range of SDS concentrations, and process II at SDS concentrations higher than CMC. The decrease of absorbance (Process I) may be explained, as in the case of cationic dyes (Pal et al., 1996; Sarkar and Podar, 2000), by the neutralization of the drug positive charges by the surfactant anions that suppresses the repulsion forces between drug molecules and favors the dimerization of the drug. The lower value of the molar absorption coefficient of the dimer ($\varepsilon_D = 7750\ M^{-1}\ cm^{-1}$) as against the molar absorption coefficient of the monomer ($\varepsilon_M = 19{,}500\ M^{-1}\ cm^{-1}$) (Rosenberg et al., 1986) sustains this assignment. Process II, the increase of absorbance, tending to a saturation level at high surfactant concentrations, can be assigned to the incorporation of the drug molecules into SDS micelles, mainly in monomer form.

The value of CMC for SDS in presence of mitoxantrone, determined from the change in the absorption spectrum of mitoxantrone (the SDS concentration corresponding to the first point marking the increase of absorbance in Fig. 3) is $CMC_{SDS} = (8.16 \pm 0.92) \times 10^{-4}$ M and was used throughout the calculations. This value is smaller than the CMC value in pure water (8.08×10^{-3} M) and that in 50 mM phosphate buffer (1.99×10^{-3} M) reported in literature (Fuguet et al., 2005), due to the well-known lowering of the surfactant CMC, by the influence of different ions and molecules present (Sarkar and Podar, 2000).

Process I was analyzed assuming a 1:2 drug:SDS interaction, using Eq. (1) (Shen et al., 1998):

$$A = \frac{A_0 + A_b K[SDS]^2}{1 + K[SDS]^2} \quad (1)$$

where A, is the measured absorbance; A_0, the absorbance of the drug in the absence of surfactant; A_b, the absorbance of the drug bound to surfactant.

The nonlinear fitting using Eq. (1) of the spectral data corresponding to process I is shown in Fig. 4a, and the results $K = (6.84 \pm 0.76) \times 10^7\ M^{-1}$ and $\varepsilon_b = 7660 \pm 436\ M^{-1}\ cm^{-1}$ are the average of three different experiments. The value of ε_b obtained for process I is in the range of the molar absorption coefficient of the drug dimer, $\varepsilon_D = 7750 \pm 550\ M^{-1}\ cm^{-1}$ (Kapuscinski and Darzynkiewicz, 1985), attesting for the increase of dimer concentration in solution, in agreement with the discussion above.

For SDS concentrations above CMC, both absorbance maxima increase but the absorbance band at 660 nm corresponding to the drug monomer becomes predominant (Fig. 2b). Also, at SDS concentration above CMC a slight red shift of both absorbance maxima can be observed (614 and 665 nm, respectively). This bathochromic shift is presumably the consequence of mitoxantrone molecule transfer from the highly polar phase (phosphate buffer) into a less polar phase (the hydrophobic core of SDS micelles). At SDS concentration higher than CMC, the increase in the absorption maxima is due to the interaction of mitoxantrone with SDS micelles. Taking into account the increase in the A_{660}/A_{610} ratio (the ratio of the absorbance at 660 nm corresponding to the drug monomer and the absorbance at 610 nm corresponding to the dimer) from 0.78 in the absence of SDS to 1.27 at SDS concentrations higher than CMC, it may be assumed that the dissociation of the mitoxantrone dimers is driven by the interaction of drug with SDS micelles. Therefore, it may be inferred that mitoxantrone molecules interact with SDS micelles as monomers.

Process II, occurring at SDS concentrations higher than CMC, was analyzed by nonlinear regression assuming a 1:1 interac-

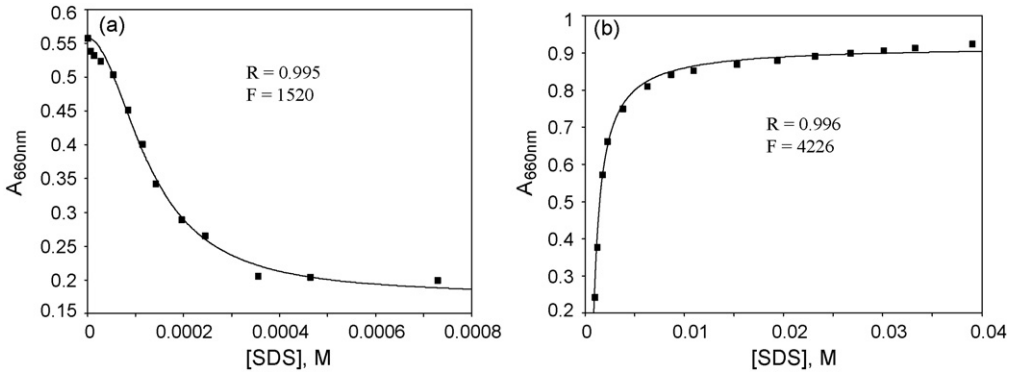

Fig. 4. Nonlinear fitting of the spectral data corresponding to: a) process I (premicellar range) using Eq. (1) and b) process II (micellar range) using Eq. (2).

tion between the drug and the SDS micelle, using the formula (2) (Fig. 4b). The results obtained are: $K = (1.14 \pm 0.05) \times 10^3\ M^{-1}$ and $\varepsilon_b = 32168 \pm 578\ M^{-1}\ cm^{-1}$.

$$A = \frac{A_0 + A_b K[SDS]}{1 + K[SDS]} \quad (2)$$

The values of the binding constant for process II indicate a relatively high affinity of mitoxantrone for anionic SDS micelles. However, the binding constants for process II are much lower than those for the electrostatic interaction between mitoxantrone and SDS anions, process I. The value obtained for ε_b is higher than the molar absorption coefficient of the monomer of mitoxantrone in aqueous medium ($19{,}500\ M^{-1}\ cm^{-1}$) due to the hydrophobic environment of the drug encapsulated in micelles.

A solute can interact with micelles in different ways: it can be adsorbed on the surface of the micelle, nonpolar molecules may be trapped in the hydrophobic core of the micelles or, in the case of molecules containing polar substituents, it can be oriented with the polar portion of the molecule situated in the surface layer and the nonpolar portion of the molecule directed into the micelle (Cudina et al., 2005). The location of molecules into micelles determines the extent of solubilization, the chemical reactivity of incorporated molecules and the rate of their release from the micelles (Kim et al., 2001). The position of the mitoxantrone molecule in the micelle can be elucidated by comparing the mitoxantrone spectra in the presence of SDS with the spectra in water and organic solvents of different polarities. The absorption spectra of mitoxantrone in protic media of different dielectric constants (water $\varepsilon = 80$, methanol $\varepsilon = 32$, ethanol $\varepsilon = 24$) present a progressive bathochromic shift of both absorption maxima on going from water to less polar solvents. Also, the A_{660}/A_{610} ratio increases with the increase of the hydrophobicity of solvents. In example in an aprotic polar solvent (dimethylsulfoxide $\varepsilon = 48$), the A_{660}/A_{610} ratio is 1.36, quite close to 1.34, the value reported for monomer mitoxantrone in polar intracellular environment (Feofanov et al., 1997). Therefore, it may be assumed that the increase in the environmental hydrophobicity induces the dissociation of the dimers of mitoxantrone, the drug being encapsulated as monomer in SDS micelles. The octanol:water partition coefficient of mitoxantrone at pH 7.4 is $\log P = 0.79$, which indicates that mitoxantrone is a fairly lipophilic drug (Burns et al., 1988). Since micelle surface is an environment with different properties from water ($\varepsilon = 36$) (Mukerjee and Ray, 1966), at SDS concentrations higher than CMC, mitoxantrone is encapsulated in surfactant micelles as monomer, and most probably situated in the micelle surface layer with its chromophore ring immersed in the micelle core and both positively charged side chains oriented towards the negatively sulfate groups of SDS, both polar and electrostatic interactions playing important role in the drug/micelle binding.

For increasing SDS concentrations, above its CMC, the absorbance at 660 nm increases asymptotically reaching the plateau when all mitoxantrone from solution is solubilized in micelles. For increasing mitoxantrone concentrations the saturation is achieved at increasing concentrations of SDS (Fig. 5).

Drug-micelle interaction can be evaluated besides the binding constant (K) by the determination of the partition coefficient (K_x), a thermodynamic parameter that represents the affinity of a given solubilizate to the micellar phase, relative to the aqueous one. The partition coefficient is important not only in elucidating the mechanism of solubilization but also in understanding of biological phenomena like interaction between drugs and biological membranes. According to the pseudo-phase model (Sepulveda et al., 1986; Kawamura et al., 1989), the partition coefficient can be

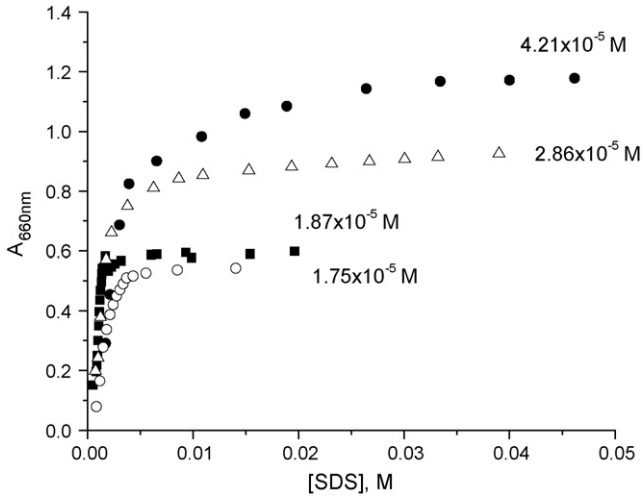

Fig. 5. Absorbance at $\lambda = 660$ nm of increasing concentrations of mitoxantrone solutions as a function of SDS concentration.

determined from the following equation:

$$\frac{1}{\Delta A} = \frac{1}{\Delta A_\infty} + \frac{n_w}{K_x \Delta A_\infty ([SDS] + C_T - CMC)} \quad (3)$$

where $\Delta A = A - A_0$, $\Delta A_\infty = A_b - A_0$ and $n_w = 55.5$ M is the molarity of water. The value of K_x is obtained from the slope of the plot of $1/\Delta A$ versus $1/([SDS] + C_T - CMC)$ as shown in Fig. 6.

The linear relation holds in a very high surfactant concentration region below which the curve tends to bend upwards with decreasing surfactant concentration. This deviation from linearity is considered to be due to the approximation made in the evaluation of Eq. (3) (Kawamura et al., 1989). The partition coefficients were evaluated for solutions containing different mitoxantrone concentrations and increasing SDS concentrations. The results are summarized in Table 1 and indicate that the partition coefficient is strongly dependent on the drug concentration. The decrease of K_x with the increase of mitoxantrone concentration indicates that solubilization of mitoxantrone in SDS micelles is a competitive process that becomes more and more difficult as the amount of drug incorporated into micelles increases.

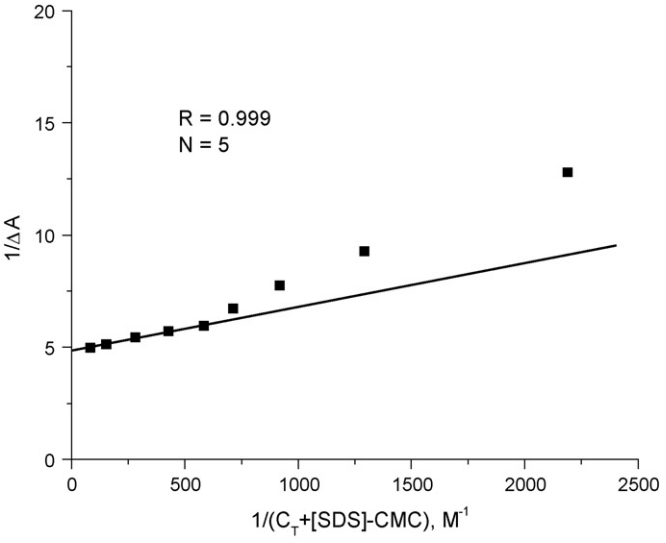

Fig. 6. Plot of $1/\Delta A$ versus $1/(C_T + [SDS] - CMC)$ for mitoxantrone (1.75×10^{-5} M) in SDS solutions. Linear regression at high SDS concentrations allows determination of the K_x values in Table 1.

Table 1
The dependence of partition coefficient (K_x) of mitoxantrone between SDS micelle and aqueous phase on drug concentration.

$C_T \times 10^5$ (M)	$K_x \times 10^{-3}$
4.21	7.85 ± 1
2.86	33.2 ± 3
1.87	118.4 ± 10

From the equation $\Delta G_x = -RT \ln K_x$, where R is the gas constant and T the absolute temperature, and K_x calculated from $K = K_x V$ (where V is SDS partial molar volume, 0.25 L/mol (Sepulveda et al., 1986)), the standard free energy change for the transfer of mitoxantrone from bulk aqueous phase to micellar phase is obtained ($\Delta G_x = -20.88$ kJ/mol). This value is in the range obtained for other opposite charge drug–surfactant systems (Cudina et al., 2005, 2008), where the interaction is predominantly electrostatic. As the surface of biological membranes frequently presents a net charge, the binding properties of charged molecules such as drugs, as well as their membrane location are very important. Therefore the binding parameters determined for the mitoxantrone–SDS system in micellar and submicellar domain are also important for the understanding of the interaction of this drug with biological membranes.

3.2. Electrochemical results

The cyclic voltammograms of mitoxantrone in phosphate buffer pH 7.4, in the absence and in the presence of SDS, are presented in Fig. 7.

In the absence of SDS the first reduction couple was analyzed by cyclic and linear RDE voltammetry. The linear plot of the peak current, I_{pc} in function of the square root of the scan rate, $\nu^{1/2}$ ($R = 0.988$, $N = 8$) attests to a diffusion wave, and from the slope a diffusion coefficient of free mitoxantrone in phosphate buffer $D_f = 8.70 \times 10^{-5}$ cm^2 s^{-1} was calculated. The standard apparent electron transfer rate, k_s, was estimated to 5×10^{-3} cm s^{-1} from the difference between the cathodic and anodic peak potential values, ΔE_{pa}, using Nicholson's formula (Bard and Faulkner, 2001). This corresponds to a quasireversible process and was assigned to the monoelectronic reduction of the drug.

The results obtained with rotating disc electrode (RDE) confirm the fact that the reduction step is monoelectronic. From the plot $E = f(\ln(i_l - i)/i))$, $E_{1/2}$ values were obtained in the absence and in the presence of SDS (graphs are not shown), and transfer coefficient α values of 0.56 and respectively 0.40 were calculated.

Gradual addition of surfactant to the mitoxantrone solution shifted the cathodic peak towards negative potential values, but the difference between the cathodic and anodic peak potential values, ΔE_p, is not modified, indicating that the electron transfer rate is not altered by the interaction with SDS. The good linearity of the plot $I_{pc} = f(\nu^{1/2})$ ($R = 0.996$) and the value of the slope of the plot $\log(I_{pc}) = f(\log \nu)$, 0.42, ($R = 0.997$) is indication that the diffusion character of the reduction wave is maintained in presence of surfactant. At the same time, a slight increase of the cathodic (negative) peak current is observed (Fig. 7a), which could be explained by the partial neutralization of the drug positive charge as consequence of the interaction with isolated SDS anions. The uncharged drug monomer-SDS complex is expected to be less solvated in polar media, and therefore its diffusion to be facilitated, meaning an increase of the diffusion coefficient as against that of the free drug in aqueous media, which results in an increase of the cathodic current. This process takes place up to a concentration of SDS around CMC and is followed by a reverse variation of the current (Fig. 7b) tending to a plateau at surfactant concentrations higher than CMC.

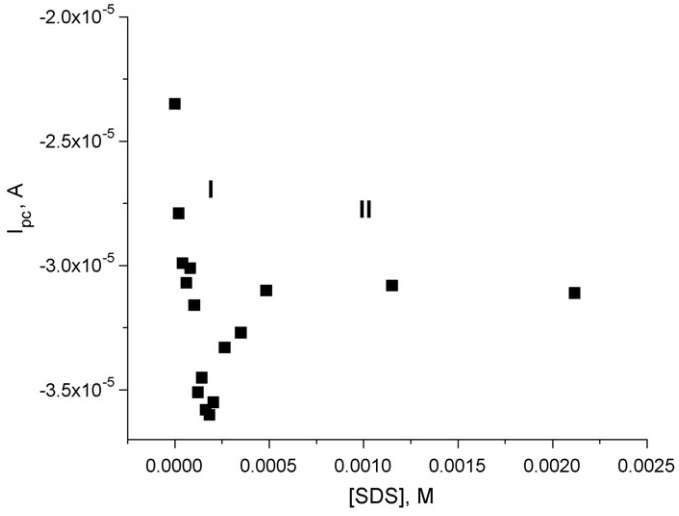

Fig. 8. Cathodic peak current dependence on SDS concentration: processes I and II are indicated.

The peak current dependence on the surfactant concentration is presented in Fig. 8 and indicates the same two processes outlined by spectral results.

The minimum of the curve corresponds to a SDS concentration of 1.85×10^{-4} M, i.e. a value smaller than the CMC determined by spectral method, probably due to the higher mitoxantrone concentration used in electrochemical experiments (about 10^{-4} M as against about 10^{-5} M), in agreement with literature data regarding the lowering of the surfactant CMC by the influence of different ions and molecules present (Sarkar and Podar, 2000). A similar behaviour was encountered for other drug–surfactant interaction

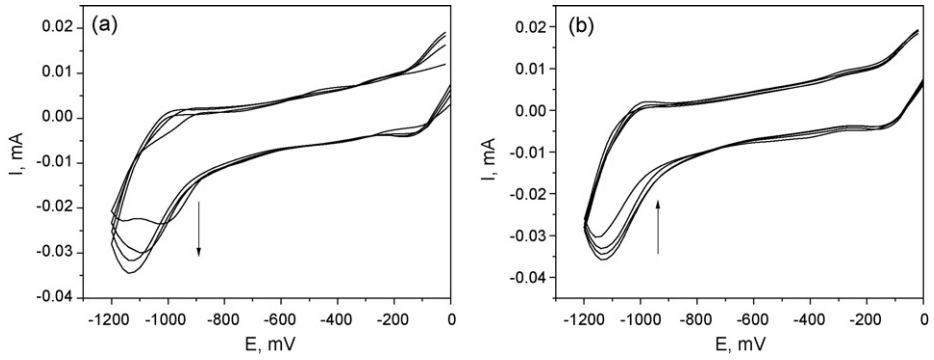

Fig. 7. Cyclic voltammograms of mitoxantrone (1.85×10^{-4} M) in phosphate buffer in the presence of different SDS concentrations: (a) $C_{SDS} = 0$–1.43×10^{-4} M; (b) $C_{SDS} = 1.43 \times 10^{-4}$–$2.44 \times 10^{-4}$ M, $\nu = 0.2$ V/s (the arrows indicate the increase (a) and (b) decrease of cathodic current in premicellar and, respectively, micellar range).

Table 2
Binding parameters for the interaction of mitoxantrone with SDS obtained by spectral and electrochemical methods.

	Spectral results		Electrochemical results			
	K (M^{-1})	ε_b (M^{-1} cm^{-1})	Potential		current	
			K (M^{-1})	p	K (M^{-1})	D_b (cm^2 s^{-1})
Process I	$(0.69 \pm 0.08) \times 10^8$	7660 ± 436	$(1.86 \pm 0.4) \times 10^8$	1.80 ± 0.14	$(1.42 \pm 0.24) \times 10^8$	$(1.04 \pm 0.3) \times 10^{-4}$
Process II	$(1.14 \pm 0.05) \times 10^3$	32168 ± 578	–	–	$(0.67 \pm 0.09) \times 10^3$	$(7.81 \pm 0.6) \times 10^{-5}$

in previous coupled electrochemical–spectral studies (Enache et al., 2007, 2008). It was found that process *I* is strongly dependent on the drug and/or surfactant charge, being absent for the interaction of mitoxantrone with positively charged surfactant (CTAB) and less evident for mitoxantrone-uncharged surfactant (Triton X-100) system (unpublished results). This can be considered as further support for the electrostatic contribution to the interaction of the drug with molecular surfactant in the premicellar range.

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

Evaluation of the binding parameters from peak current measurements is based on the variation of the free drug concentration and diffusion coefficient, as consequence of the interaction.

The peak current for a reversible electron transfer at 25 °C is given by:

$$I = 2.69 \times 10^5 n^{3/2} A D_O^{1/2} C_T \nu^{1/2} \quad (4)$$

where n is the number of electrons transferred, A the electrode area and ν is the sweep rate.

The total current at any SDS concentration is given by:

$$I = B[D_f^{1/2} C_f + D_b^{1/2} C_b] \quad (5)$$

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

$$I = B[D_f^{1/2} C_T - (D_f^{1/2} - D_b^{1/2}) C_b] \quad (6)$$

where B represents all the constants ($2.69 \times 10^5 n^{3/2} A \nu^{1/2}$) 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 surfactant ($I_0 = B D_f^{1/2} C_T$), Eq. (6) becomes:

$$I = I_0 - B(D_f^{1/2} - D_b^{1/2}) C_b \quad \text{and} \quad C_b = \frac{I_0 - I}{B(D_f^{1/2} - D_b^{1/2})} \quad (7)$$

On another side, for 1:1 and respectively for 1:2 interactions, if $C_b \ll$ [SDS], the equilibrium constant K is given by:

$$K = \frac{C_b}{(C_T - C_b)[\text{SDS}]} \quad \text{and} \quad K = \frac{C_b}{(C_T - C_b)[\text{SDS}]^2} \quad (8)$$

where [SDS] at equilibrium may be approximated by the total concentration of SDS. Combining Eqs. (7) and (8) and noting I_{inf} the current corresponding to a solution containing SDS in excess ($I_{inf} = B D_b^{1/2} C_b$), the current expression for a 1:1 interaction becomes:

$$I = \frac{I_0 + I_{inf} K[\text{SDS}]}{1 + K[\text{SDS}]} \quad (9)$$

and for a 1:2 interaction:

$$I = \frac{I_0 + I_{inf} K[\text{SDS}]^2}{1 + K[\text{SDS}]^2} \quad (10)$$

Both processes were analyzed by nonlinear regression using Eqs. (9) and (10), and the results are presented in Table 2, together with the absorption spectroscopy results.

The stoichiometry and binding parameters can also be determined from potential measurements using cyclic or linear (RDE) voltammetry. If the oxidized form of the drug is stabilized by the interaction, the potential of the redox couple is shifted towards negative values and the interaction constant (K) and the number of ligands (p) may be determined using the following equation (Bard and Faulkner, 2001):

$$E_{1/2}^{complex} - E_{1/2}^0 = -\frac{RT}{nF} \ln K - \frac{pRT}{nF} \ln[\text{SDS}] \quad (11)$$

The half-wave potential in the absence ($E_{1/2}^0$) and in the presence ($E_{1/2}^{complex}$) of different surfactant concentrations was evaluated from cyclic voltammetry as $(E_{pc} + E_{pa})/2$, or from RDE experiments. Analysis of the data for SDS concentrations in the premicellar range, (process I, Fig. 8) according to Eq. (11), allows determining the binding constant, $K = (1.86 \pm 0.4) \times 10^8$ M^{-1}, and, for $n = 1$, as obtained from RDE experiments, $p = 1.80 \pm 0.14$, attesting for a 1:2 drug–SDS complex at SDS concentration smaller than CMC (Fig. 9).

Analysis of the data in Table 2 shows a reasonable agreement between the binding constants determined by electrochemical and spectral methods for both processes. However, higher errors are noted for electrochemical methods (13–20%) comparatively with spectral ones (4–13%). Electrochemical methods support the 1:2 stoichiometry of interaction in the premicellar range of SDS concentrations, in agreement with literature data, based on other methods (Liu et al., 2007). The diffusion coefficients were calculated both from the slope of the current dependence on the scan rate in the absence and presence of surfactant below and above CMC, and from the nonlinear fitting of both processes. However, although the results account for the variation of the current during processes I and II in Fig. 8, the differences between D_f and D_b corresponding to processes I and II are small, and the experimental errors are important, mainly due to the possible interference of adsorption of the drug on the electrode.

The advantage of using coupled spectral and electrochemical methods in the study of the drug–surfactant interaction resides in

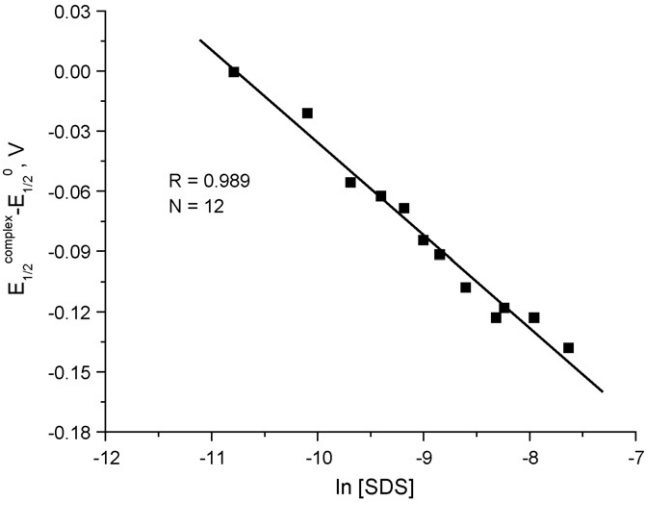

Fig. 9. Plot of the difference between the half-wave potential in the presence ($E_{1/2}^{complex}$) and in the absence of SDS ($E_{1/2}^0$) against the logarithm of the SDS concentration (Eq. (11), $\nu = 0.2$ V/s).

the complementarity of the information obtained. Electrochemical studies give information regarding the complexation ratio, the diffusion coefficients of the free and bound drug, whereas the spectral ones, more specific from a structural point of view, allow the determination of molar absorption coefficient of the different species involved. Both types of methods allow a quantitative evaluation of the strength of interaction, even in another range of drug concentration. It is also to be noted that the differences observed between the electrochemical and spectral results may be due to the higher concentration (about an order of magnitude) used in cyclic voltammetry as against absorption spectroscopy. At these concentrations autoassociation of the drug is important and aggregation beyond the dimer may occur, and these species were not considered in the present treatment of experimental data.

4. Conclusions

The interaction of antitumor drug mitoxantrone with anionic surfactant sodium dodecyl sulfate has been investigated in premicellar and micellar concentration range using coupled electrochemical methods with absorption spectroscopy. Both absorption and cyclic voltammetry results indicate two distinct interaction types between mitoxantrone and SDS, dependent on the surfactant concentration: process I in premicellar range, assigned to the electrostatic interaction between the positively charged groups of the drug and the negatively charged surfactant group; and process II in micellar range when the surfactant micelles are formed and the drug is encapsulated in micelles in monomer form. Information about the position of mitoxantrone into SDS micelles was obtained by analyzing the absorption spectra of the drug in solvents with different polarities. The results indicated that mitoxantrone molecule is most probable located in the micelle surface layer with its chromophore immersed in the micelle core and both positively charged side chains oriented towards the negatively sulfate groups of SDS, both polar and electrostatic interactions playing important role in the binding of drug to SDS micelles. The results are important for the possible utilization of surfactant micelles as drug carriers, as well as for the understanding of the interactions of drugs with biological membranes.

Acknowledgements

CNCSIS financial support (grant 486/2009) is gratefully acknowledged.

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