ELECTROCHEMICAL STUDY OF THE INTERACTION OF DOXORUBICIN WITH ANIONIC SURFACTANTS (SODIUM LAURYL SULFATE)

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abstract: The interaction between an antitumoral drug, doxorubicin, and an anionic surfactant, sodium lauryl sulfate (SDS), is investigated by cyclic voltammetry. The cyclic voltammograms recorded in phosphate buffer (pH=7.09) in the presence of SDS indicate a shift of the peak potentials of the first monoelectronic reversible couple towards negative values and a decrease of the cathodic peak current. Using non-linear fitting of the experimental data, values of the binding constant, K and 1:1 stoechiometry of the interaction were determined. The results outline a weak predominantly electrostatic drug – surfactant interaction in the range of pre-micellar and micellar concentrations of surfactant (SDS).

Introduction

Doxorubicin (Fig. 1) is one of the most potent anthracyclinic antitumoral drugs, the action of which is due to the intercalation of the aromatic moiety of the drug between the DNA base pairs, resulting in the inhibition of the transcription by blockage of RNA polymerase [1 – 3]. Its mode of action depends critically on the concentration of drug in the tumoral cell.

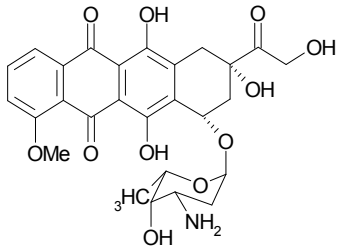

Fig.1 Molecular structure of doxorubicin

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The principal way of penetrating of the drug into the cell is the passive diffusion of the uncharged drug through the plasmatic membrane. However, the secondary effects associated with chemotherapy limit the dose employed in the treatment of different types of tumors and may cause drug resistance. Therefore, in the last years different transport systems were employed, like nanoparticles or liposomes, which ensure the necessary intracellular concentration and prevent the degradation of the drug by interaction with plasmatic proteins [4 - 6]. The use of liposomes as drug carriers makes necessary the study of the interaction of drugs with micellar systems, implying the elucidation of the nature of these interactions. Previous studies of the inclusion of adriamycin into polymeric micelles have pointed out that electrostatic and hydrophobic interactions may play an important role in ensuring an enhanced accumulation in the tumoral tissue and a higher *in-vivo* antitumoral activity [7].

Therefore the aim of the present paper is to investigate the interaction of the antitumoral drug doxorubicin with an anionic surfactant, sodium lauryl sulfate (SDS) by cyclic voltammetry, in order to evidence the drug species involved and to elucidate the nature of the interactions in the submicellar and micellar range of concentrations. If spectral methods are usually employed in the study of intermolecular interactions, the use of electrochemical methods in this type of studies is much more recent, the first papers being published only in the last years [8, 9].

Experimental

Doxorubicin (hydrochloride form) was obtained from Sigma. It was dissolved in phosphate buffered saline (pH=7.09) in a concentration range 10^{-4}–10^{-2}M.

Tetrabutylammonium bromide (0.1M) was used as background electrolyte in phosphate buffered saline to stabilize the micelles and to hinder their aggregation.

Sodium lauryl sulfate (SDS) (10^{-4}-10^{-1}M) was dissolved in solution containing 0.1M tetrabutylammonium bromide.

Cyclic voltammetry experiments were performed in a three electrodes cell: one platinum electrode as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode as reference electrode; the voltammograms were recorded using a Voltalab 32 electrochemical combine.

Results and discussion

The electrochemical reduction of doxorubicin in dimethyl sulfoxide (DMSO) supposes two mono-electronic transfer steps [10]. We have started by studying only the first wave in aqueous medium (phosphate buffered saline, pH=7.09) for a potential range -1000 - 0mV at different sweep rates in the absence and in presence of SDS. Literature data indicate that in aqueous solution at pH~7, doxorubicin is positively charged. The cyclic voltammograms recorded at a sweep rate of $0.1Vs^{-1}$ in the absence and in presence of different amounts of SDS are presented in Fig. 2.

The gradual addition of surfactant to the aqueous solution of doxorubicin shifted the cathodic peak towards negative potential values and the anodic peak towards more positive potential values so that the difference ΔE_p increases with the added SDS concentration (Fig. 2 and Table 1).

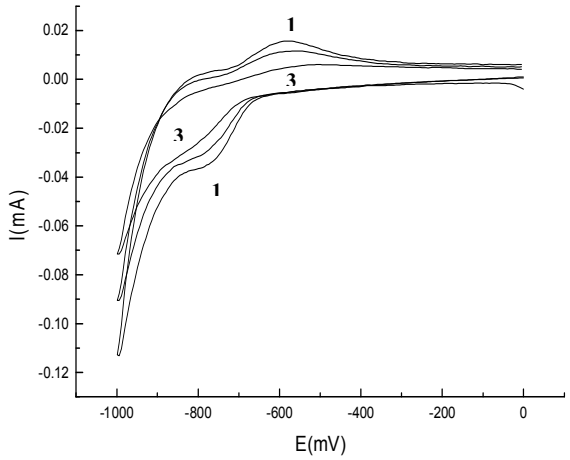

Fig. 2 *Cyclic voltammogram of a solution of doxorubicin (c = 1*10^{-4}M) in DMSO. 1 - c_{SDS} = 3.8*10^{-3}M, 2 - c_{SDS} = 9.26*10^{-3} M, 3 - c_{SDS} = 13.44*10^{-3} M*

The relevant electrochemical data for different SDS concentrations are presented in Table1:

Table 1 Electrochemical data for the first wave (v=100mV/s)

[SDS] (mM)	E_{pc} (mV)	I_{pc} (mA)	E_{pa} (mV)	I^0_{pa} (mA)	E^0_{sp} (mV)	I^0_{sp} (mA)	$\Delta E = E_{pa}-E_{pc}$ (mV)	$E_{1/2}=(E_{pa}+E_{pc})/2$ (mV)
0	-781	-0.0247	-639	0.0124	-1000	-0.108	142	-710
4.79	-790	-0.0352	-602	0.0154	-1000	-0.119	188	-696
5.7	-799	-0.0346	-602	0.0154	-1000	-0.113	197	-700.5
6.61	-807	-0.0363	-610	0.016	-1000	-0.122	197	-708.5
7.5	-822	-0.0346	-602	0.0132	-1000	-0.107	220	-712
9.26	-842	-0.0352	-581	0.015	-1000	-0.0897	261	-711.5

This ΔE_p variation reflects the diminution of the electron transfer rate estimated according to Nicholson's relationship from the corresponding Ψ function values [11]:

$$\Psi = \gamma^{\alpha/2} * \frac{(R*T)^{1/2} * k_f}{(n*F*D_{ox}*\pi*\upsilon)^{1/2}}, \text{ with } \gamma = \frac{D_{ox}}{D_{red}} \approx 1 \qquad (1)$$

where D_{ox} and D_{red} are the diffusion coefficients of the oxidized and reduced species, respectively, α – the transfer coefficient, n – the number of the electrons and ν - the sweep rate.

In the presence of SDS the standard transfer rate decreases from $2.7*10^{-3}$ to $0.92*10^{-3}$cm/s as a consequence of encapsulation of the drug into the surfactant micelles.

The experimental data point out also a shift of the half-wave potential (calculated as $E_{1/2}=(E_{pa}+E_{pc})/2$) towards more negative values attesting for the interaction between the positively charged drug and the anionic surfactant. The small $E_{1/2}$ variation suggests a rather weak drug-surfactant interaction.

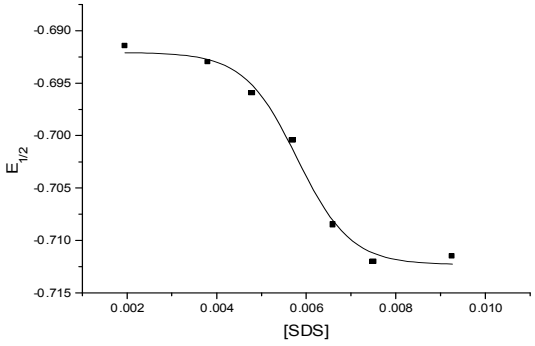

Fig.3 *Dependence of the half-wave potential on the surfactant concentration*

The reversible reduction of the drug - SDS aggregate may be accounted for by the two equlibria:

$$M + e^{-} = M_{red}$$
$$M + pL = ML_p$$

where L stands for the surfactant (SDS) and M stands for the electroactive drug (doxorubicin). The presumption of reversibility requires that these equlibria be respected simultaneously. For a reversible wave the half-wave potential is very close to $E^{0'}$ and may be evaluated as $(E_{pa}+E_{pc})/2$.

Knowing the half-wave potentials in the absence ($E_{1/2}^{0}$) and in presence ($E_{1/2}^{complex}$) of the complexing agent, the complexation constant and the number of ligands may be determined using the following equation [11]:

$$E_{1/2}^{complex} - E_{1/2}^{0} = -\frac{RT}{nF}\ln K - \frac{pRT}{nF}\ln c_X \qquad (2)$$

So plotting the variation of the half-wave potential ($\Delta E_{1/2}$) against the logarithm of the surfactant concentration we calculated the complexation constant value, $K=295M^{-1}$, and the number of the ligands, p=1.025. The results attest for a 1:1 drug - SDS complex.

The sigmoidal plot (Fig. 3) allows determining the concentration value corresponding to the inflexion point, $5.8*10^{-3}$M. This value is in the range of the critical micellar concentration for SDS, a fact that can be explained by the enclosure of the drug into the micelles and consequently the change of the aqueous environment for a non-polar environment in micelles [12].

Appling the formula for the critical micellar concentration (CMC) [13] for different electrolyte concentrations,

lg CMC = -3.65 – 0.57 * lg [electrolyte]

we found that in our experiments the range of the CMC is $4.48*10^{-3}$M – $6.41*10^{-3}$M. These values are lower than the true CMC of SDS ($8.0*10^{-3}$M) in the aqueous solution. A possible explanation is that the electrostatic repulsion within the anionic moiety of SDS is reduced by the positive charge of the added drug cation [14] and attests for the predominant electrostatic nature of the drug – SDS interaction.

The experimental data were fitted by a non-linear equation (Fig. 4) assuming a 1:1 interaction between the drug and SDS and the bound species concentration smaller than the ligand initial concentration:

$$M + L = ML;$$
$$K = \frac{c_{ML}}{c_M * c_L} = \frac{c_b}{(c_M^0 - c_b) * c_T^{SDS}} \quad (3)$$

so $c_b = \frac{K * c_M^0 * c_T^{SDS}}{1 + K * c_T^{SDS}}$, where c_M^0 is the initial drug concentration and c_T^{SDS} is the SDS total concentration.

The current peak expression for a reversible wave is given by:

$$I = 2.69 * 10^5 * n^{3/2} * A * D_f^{1/2} * c_M^0 * v^{1/2} \quad (4)$$

where n is the electrons number, A the electrode surface and ν the sweep rate.

In the complexation case we can write:

$$I = B * [D_f^{1/2} * c_M^0 - (D_f^{1/2} - D_b^{1/2}) * c_b] \quad (5)$$

where B represents all the constants and D_f and D_b are the diffusion coefficients of the free and bound drug, respectively. Replacing the c_b concentration determined from the equilibrium eq. (3), the current I_0 in the absence of ligand ($I_0 = B * D_f^{1/2} * c_M^0$) and the

current I_{inf} corresponding to a solution containing SDS in excess ($I_{\inf} = B * D_b^{1/2} * c_b$) the current expression becomes:

$$I = \frac{I_0 + I_{\inf} * K * c_T^{SDS}}{1 + K * c_T^{SDS}} \tag{6}$$

The dependence of the cathodic peak current on the SDS concentration is presented in Fig.4:

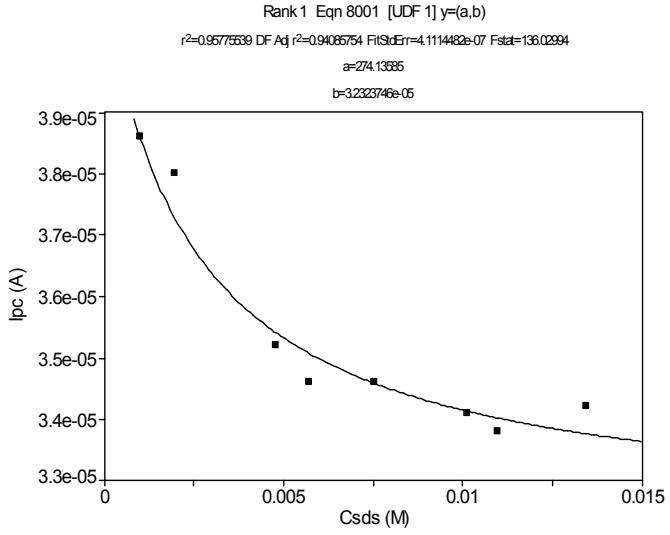

Fig.4 *The dependence of the cathodic peak current on the SDS concentration*

The value obtained for the current is I_{inf} = -3.23*10^{-5}A. For the binding constant K the result obtained, 274M^{-1}, is in good agreement with the value determined from the $E_{1/2}$ variation.

Plotting the ratio between the peak current value in the presence (I_{pc}) and in the absence of SDS (I_o) against the P/D ratio, a value of about 40 was obtained for the P/D ratio at the inflexion point (Fig. 5). This value corresponds to the critical micellar concentration determined from the other plots (5*10^{-3}M) for the drug concentration used in cyclic voltammetry experiments.

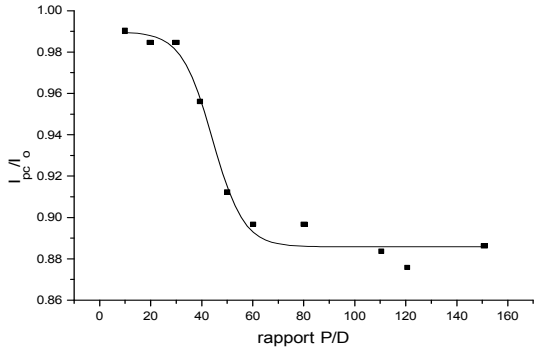

Fig.5 *Dependence of the ratio between the peak current value in the presence (I_{pc}) and in the absence of SDS (I_o) on the P/D ratio*

The explanation of this behavior may be as follows: for SDS concentrations in the pre-micellar range there is a neutralization process between the drug's positive charge and the surfactant's negative charge that can account for the cathodic peak shift towards more negative potential values in case of voltammetry. Increasing the SDS concentration added to the doxorubicin solution, the surfactant micelles are formed and the drug is encapsulated in the micelles, preferentially in the monomeric form. The observed decrease of the cathodic peak current towards the I_{inf} limit corresponds to the lower diffusion coefficient of the drug – containing aggregates.

The electrochemical results obtained by cyclic voltammetry evidence the predominant electrostatic nature of the doxorubicin – SDS interaction. However, the parameters that characterize the interaction are rather estimative values because of the experimental limits of the electrochemical methods that require a drug concentration range of 10^{-4}-10^{-3}M when the auto-aggregation processes of the drug become important.

Therefor, in order to get a deeper understanding of the character of the drug – surfactant interactions, the use of spectral methods in conjunction with electrochemical ones is required and is in progress in our laboratory.

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