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Research Paper

Spectroscopic investigations of the molecular interaction of anticancer drug mitoxantrone with non-ionic surfactant micelles

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Keywords
absorption spectroscopy; binding constant; mitoxantrone; non-ionic surfactant micelles; partition coefficient

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Abstract

Objectives The aim of this study was to investigate the interaction of the anticancer drug mitoxantrone with non-ionic micelles, as simple model systems of biological membranes.

Methods UV-VIS absorption spectroscopy was used to quantify the drug–surfactant micelle interactions in terms of the binding constant and the micelle–water partition coefficient of the drug.

Key findings Interaction of mitoxantrone with non-ionic micelles reduces the dimerization process of mitoxantrone, the drug molecules being encapsulated into micelles as monomer. The strength of the interaction between mitoxantrone and non-ionic micelles is higher at pH 10 than at pH 7.4, and depends on the surfactant in the order Tween 80 > Tween 20 > Triton X-100. The higher partition coefficient at pH 10 compared to pH 7.4 suggests that at basic pH the deprotonated mitoxantrone is incorporated more efficiently into the hydrophobic medium of non-ionic micelles compared to physiological pH, when the protonated drug is predominant.

Conclusions These results on simple model systems miming the drug–membrane interactions contribute to the elucidation of the behaviour of the drug *in vivo*, as well as the possible utilization of surfactant micelles as drug carriers.

Introduction

Physico-chemical aspects of the binding of drugs from different therapeutic categories to model and natural membranes (micelles of surfactants, phospholipid bilayers, erythrocyte ghosts, etc.) have been the subject of extensive studies.[1–5] Because biological membranes are extremely complex multicomponent structures, surfactant micelles with much less complexity have been used as model systems for biomembranes to investigate different aspects of bilayer properties and functions.[6,7] Surfactant micelles have also been widely utilized to increase the solubility of hydrophobic drugs, which is a problem in the formulation of an acceptable dosage form.[8] Drugs may be solubilized in the hydrophobic core and/or on the interface of the micelles. The predominant location of the drug depends on its hydrophobicity and interactions with the surfactant.[9] The extent of the drug–surfactant interaction 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).[10]

The selective biodistribution of an ionic drug in tissues and membranes depends on its self-aggregation and complex interactions with the molecular surroundings. Sodium dodecyl sulfate (SDS), cetyltrimetylammonium bromide (CTAB), Triton X-100, Brij-35 and Tweens are commonly accepted as model systems for studying different aspects of membrane interactions with drug molecules, including their localization.[1–4]

As many biological processes occur at the ionizable surface of membranes or along their hydrophobic region, a comparative study of the drug interaction with cationic, zwiterionic, anionic and neutral surfactants may provide useful information on the nature of the drug–membrane interaction.

Mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)-amino]-ethyl]-amino]-9,10-anthracenedione) is a synthetic anthracenedione antitumour drug developed in order to find a cytotoxic agent with decreased cardiotoxicity compared with doxorubicin. Mitoxantrone has shown significant clinical effectiveness in the treatment of advanced breast and prostate

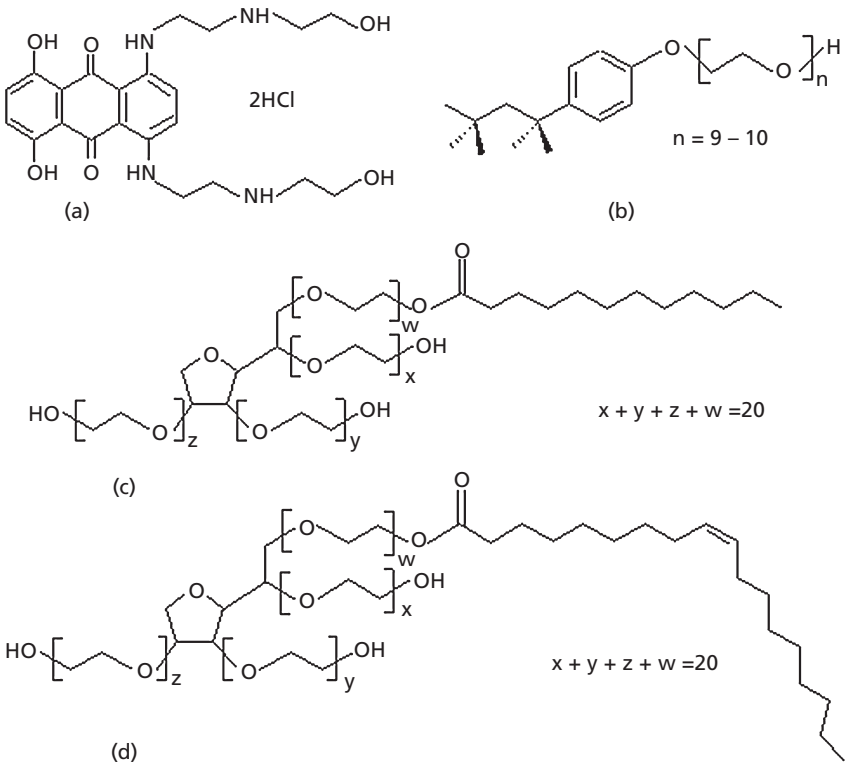

Figure 1 Chemical structures of (a) mitoxantrone, (b) Triton X – 100, (c) Tween 20 and (d) Tween 80.

cancers, lymphoma and acute leukemia.[11–13] Mitoxantrone is a DNA intercalating agent and topoisomerase II inhibitor that causes DNA strand breaks.[14] Previous studies suggest that mitoxantrone has less carditoxicity than doxorubicin, but further investigations reveal that cardiotoxicity can occur at any time during therapy, and the risk increases with increased cumulative dose.[15,16] Different drug-delivery systems have been studied in an attempt to improve the antitumour effect of mitoxantrone and to prevent harmful side effects.[17–20] In this context, the utilization of micelles as drug carriers presents some advantages compared with other alternatives, such as soluble polymers and liposomes: micelles can solubilize poorly soluble drugs and thus increase their bioavailability, stay in the body (blood) long enough to provide gradual accumulation in the required area, their size permits them to accumulate in areas with leaky vasculature, specific ligands can be attached to their surface in order to optimize the controlled release and specificity of pharmacological effect can be obtained in an easy and reproducible manner on a large scale.[21]

The structure of mitoxantrone is shown in Figure 1a. It has a planar heterocyclic ring substituted with two nitrogen-containing side chains, positively charged at physiological pH.

In our previous work we have reported the interaction of mitoxantrone with anionic (SDS)[22] and cationic (CTAB)[23] surfactants in a premicellar and micellar range of concentrations. Anionic and cationic micelles were chosen as a model of the lipid system in order to study the contribution of different charges at the polar surfactant head groups (i.e. the electrostatic contribution) to the drug binding. To further understand the nature of the interaction between mitoxantrone and micellar systems, in the present work the interaction between mitoxantrone and the non-ionic surfactants Triton X-100, polyoxyethylene (20) sorbitan monolaurate (Tween 20) and polyoxyethylene (20) sorbitan monooleate (Tween 80) (Figure 1b–d) was investigated, in order to clarify the hydrophobic contribution to the drug binding. The drug–non-ionic micelle interactions were investigated in submicellar and micellar concentration ranges, using UV-VIS absorption spectroscopy. The experiments were performed at pH 7.4 and pH 10, taking into account the fact that the biological activity of mitoxantrone is susceptible to changes in the equilibrium between protonated and deprotonated forms of the drug. At pH 7.4 mitoxantrone exists as a dication, whereas at pH 10 it is uncharged. The absorption measurements were used to quantify the drug–surfactant micelle binding constants and micelle–water partition coefficients of the drug by applying the mathematical models that consider partitioning of the dye between the micellar and aqueous pseudo-phases.

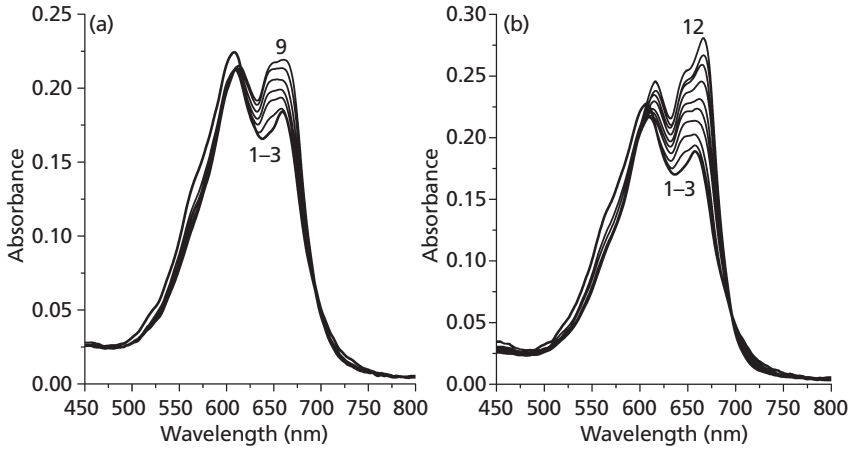

Figure 2 Visible absorption spectra of mitoxantrone (1.25×10^{-5} M) at pH 7.4 at different concentrations of (a) Triton X-100 (0–8.42×10^{-4} M, spectra 1–3; 1.70×10^{-3} M to 2.56×10^{-2} M, spectra 4–9) and (b) Tween 20 (0 to 2.63×10^{-4} M, spectra 1–3; 4.13×10^{-3} M to 3.12×10^{-2} M, spectra 4–12).

Materials and Methods

Mitoxantrone and the surfactants Triton X-100, Tween 20 and Tween 80 were analytical grade, supplied by Sigma and used as received. Experiments were performed at room temperature and double-distilled water was used for the preparation of solutions. Visible absorption spectra were recorded on a Unicam Helios-α spectrophotometer with a matched pair of quartz cuvettes of 1 cm optical length. Mitoxantrone concentrations (1.10×10^{-5} to 1.38×10^{-5} M) in phosphate buffer solution (pH 7.4, ionic strength 0.15 M) were determined spectrophotometrically at 660 nm, using the molar absorption coefficient $\varepsilon = 19\,500$/M/cm.[24] Mitoxantrone–surfactant micelles binding constants and micelle–water partition coefficients were determined from the absorbances at $\lambda = 660$ nm of a series of solutions containing a fixed drug concentration and increasing surfactant concentrations (2.00×10^{-5} to 4×10^{-2} M), absorption measurements being made after 1–2 min, time sufficient to ensure the attainment of equilibrium. The spectral results are the average of three to five different experiments. To determine the critical micellar concentration (CMC) of all three surfactants in the presence of mitoxantrone, the change in the absorption spectra of the drug, which indicates the beginning of micelle formation, was used.[25] At low surfactant concentrations, no variation in absorbance was observed and the onset of increased absorbance with further addition of surfactant was considered to be the CMC.[26,27] Linear and non-linear fitting of the experimental data were performed using Origin 7.0 and Table Curve 2D v5.01 software.

Results and Discussion

The absorption spectra of mitoxantrone in phosphate buffer pH 7.4 in the presence of different Triton X-100 and Tween 20 concentrations are presented in Figure 2a and b. In phosphate buffer pH 7.4, mitoxantrone exhibits two absorption bands at 660 and 610 nm, and a shoulder at about 560 nm, more evident at higher drug concentrations. Previous results indicated that the shape of the absorption spectrum of cationic dyes and drugs is dependent on concentration and this dependence is usually assigned to the formation of molecular aggregates.[28–30] Accordingly, in our previous work on mitoxantrone the band at 660 nm was assigned to the monomer (M), the band at 610 nm to the dimer (D) and the band around 560 nm to the formation of the higher aggregates of the drug.[31]

On the addition of all non-ionic surfactants at concentrations lower than their CMC to mitoxantrone solutions no spectral changes were observed and the absorbance of mitoxantrone remained almost constant (Figure 2a and b, spectra 1–3). When the concentration of surfactants was increased above CMC, the maximum absorption bands of mitoxantrone are shifted towards longer wavelengths (Table 1) and the absorbance of monomer band at 660 nm increases. The increase in monomer absorbance with increasing surfactant concentration above CMC is due to the interaction of mitoxantrone with surfactant micelles. Unlike the previously investigated SDS[22] and CTAB[23] micelles, a shoulder of the monomer band appears around 647 nm in the case of interaction with non-ionic micelles. This splitting of the monomer band can be due to the ionic–hydrophobic interactions between the positively charged –NH groups on chains of the drug at pH 7.4 and the hydrophobic part of the surfactant micelles. Strong ionic–hydrophobic interactions were observed in the case of CTAB micelles, but between the uncharged ring system of the drug and the cationic headgroups of surfactant.[23] Taking into account an increase in the ratio of the monomer and dimer absorbances, AM/AD from

Table 1 Spectral parameters (absorption maxima of dimer – λ_D, monomer – λ_M, and the experimental ratio of monomer to dimer absorbances – A_M/A_D) of mitoxantrone in the presence of micellar solutions of Triton X-100, Tween 20 and Tween 80, at pH 7.4 and pH 10

	pH 7.4			pH 10		
Mitoxantrone	λ_D (nm)	λ_M (nm)	A_M/A_D	λ_D (nm)	λ_M (nm)	A_M/A_D
Buffer	610	660	0.76	614	666	0.68
Triton X-100 (0.022 M)	615	666	1.07	623	676	1.36
Tween 20 (0.025 M)	617	667	1.15	624	676	1.36
Tween 80 (0.025 M)	618	669	1.19	624	676	1.38

0.76 in the absence to 1.07, 1.15 and 1.19 in the presence of micellar concentrations of surfactants (Table 1), the dissociation of the dimers and higher aggregates triggered by the interaction of mitoxantrone with surfactant micelles can be assumed. Encapsulation of drug molecules into micelles in monomer form can be relevant from a therapeutic point of view, since the dose of antitumour drugs used clinically is generally more than tens of micromolar concentration[32] and at these concentrations aggregation occurs, affecting transport across bilayer lipid membrane and consequently influencing the antitumour action.[33] Also, the formation of drug aggregates can result in highly localized concentrations at the target sites associated with local toxicity and/or lowered bioavailability.

The interaction of the drug with non-ionic micelles is characterized by the isosbestic point at 698 nm, supporting the formation of a 1 : 1 drug–micelle complex at surfactant concentrations above CMC.

The influence of pH on the mitoxantrone–non-ionic micelles interaction was also investigated and the results are summarized in Table 1 and Figure 3. Mitoxantrone is a weakly basic drug with two ionizable amines with pK_a values of 8.3–8.6, therefore its distribution will be affected by the microenvironmental pH. Literature data show that bicarbonate-induced alkalinization enhances the antitumour effects of mitoxantrone in different tumour model systems[34,35] and in cancer cells the protonated species seem to bind at cellular constituents with greater affinity than the deprotonated forms, while the latter appear to cross more easily through the membranes.[36] We therefore studied the interaction of mitoxantrone with non-ionic surfactants at pH 7.4 where mitoxantrone exists as dication with two positive charges on the aliphatic side chains,[24] and at pH 10, where mitoxantrone is uncharged due to the deprotonation of amino groups of side chains.[37]

At pH 10, both absorbance maxima of the free drug are red shifted (666 and 614 nm, respectively) and the A_M/A_D ratio decreases slightly from 0.76 to 0.68, indicating that the dimerization process is favoured in a basic environment. At basic pH, deprotonation of NH_3^+ groups of the side chains reduces the repulsion between monomers and favours the dimerization process.[37] In the presence of non-ionic micelles, the spectral behaviour of mitoxantrone in carbonate buffer pH 10 is quite similar for all the surfactants used: the maximum absorption bands of mitoxantrone are shifted towards longer wavelengths (Figure 3, Table 1) and the absorbance of monomer and dimer bands increases.

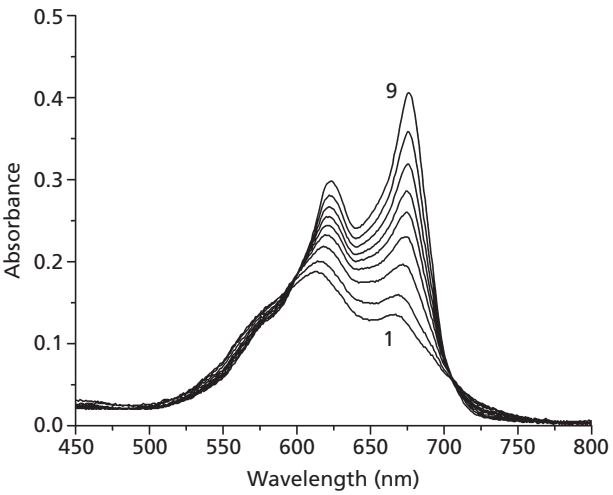

Figure 3 Visible absorption spectra of mitoxantrone (1.25×10^{-5} M) at pH 10 at different concentrations of Triton X-100: 0, curve 1; 3.00×10^{-2} M, curve 9.

The variation of the absorbance at 660 and 610 nm as a function of surfactant concentration at pH 7.4 and pH 10 is presented in Figure 4. It can be observed that for all three surfactants the absorbance of the monomer peak increases with surfactant concentration up to a concentration of approximately 0.03 M at pH 7.4 and approximately 0.01 M at pH 10; above these concentrations the absorbance seems to reach a limiting value and becomes almost constant. At the same time, for Tween 20 and Tween 80 the absorbance of dimer band at 610 nm presents initially a slight decrease in the range of premicellar aggregation of surfactant and at concentrations higher than 0.005 M it starts to increase; for Triton X-100 the behaviour is different from Tweens, i.e. the absorbance at 610 nm presents initially a slight decrease, than remains almost constant and at Triton X-100 concentrations higher than 0.015 M it starts to decrease.

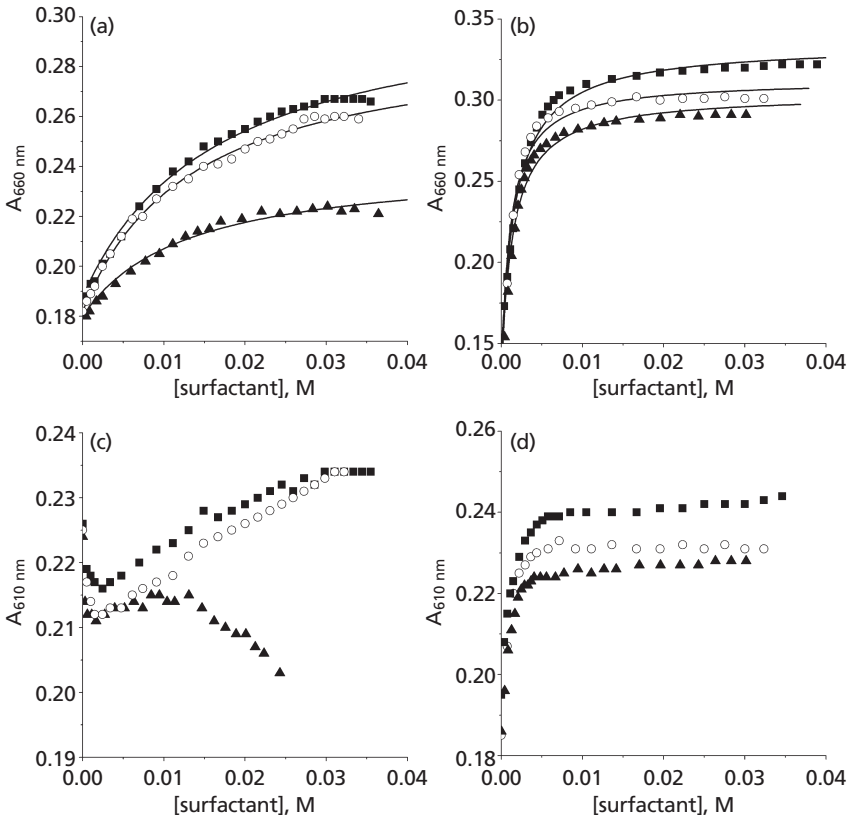

Figure 4 The monomer ($A_{660\ nm}$) and dimer ($A_{610\ nm}$) absorbance change of mitoxantrone as a function of Triton X-100 (▲), Tween 80 (o) and Tween 20 (■) surfactant concentration at pH 7.4 (a and c) and pH 10 (b and d). The symbols represent the experimental data and the full lines are the results of non-linear fitting using equation (1).

Table 2 Binding constant (K_b), partition coefficient (K_x), the Gibbs free energy of binding (ΔG_b^0) and the standard free energy change for the transfer of mitoxantrone from bulk water to micellar phase (ΔG_x^0) for the interaction of mitoxantrone with Triton X-100, Tween 20 and Tween 80 micelles at pH 7.4 and pH 10

	pH 7.4				pH 10			
Surfactant	K_b, M^{-1}	ΔG_b^0, kJ mol^{-1}	K_x	ΔG_x^0, kJ mol^{-1}	K_b, M^{-1}	ΔG_b^0, kJ mol^{-1}	K_x	ΔG_x^0, kJ mol^{-1}
Triton X-100	30 ± 2	–8.43	$(8.31 \pm 0.4) \times 10^3$	–22.34	472 ± 63	–15.25	$(1.33 \pm 0.2) \times 10^5$	–29.22
Tween 20	52 ± 5	–9.79	$(1.73 \pm 0.2) \times 10^3$	–18.40	610 ± 34	–15.89	$(3.64 \pm 0.4) \times 10^4$	–26.01
Tween 80	71 ± 4	–10.56	$(3.11 \pm 0.3) \times 10^3$	–19.91	798 ± 83	–16.56	$(5.61 \pm 0.3) \times 10^4$	–27.08

The values of CMC for all surfactants in the presence of mitoxantrone, determined from the change in the absorption spectrum of mitoxantrone (the surfactant concentration corresponding to the first point marking the increase of absorbance in Figure 4a), are $(1.70 \pm 0.25) \times 10^{-3}$ M, $(4.13 \pm 0.58) \times 10^{-4}$ M and $(2.94 \pm 0.31) \times 10^{-4}$ M for Triton X-100, Tween 20 and Tween 80, respectively. These CMC values are different from those in pure water because the presence of an organic compound may change the CMC surfactants, therefore the CMC values determined from the change in absorption spectrum of mitoxantrone have been considered in the calculations.[27]

The thermodynamic process of the interaction of drugs with surfactants and the transfer of the drug between bulk water and micellar phases is characterized by free energy changes, binding constant and partition coefficient. The interaction of mitoxantrone–non-ionic surfactant micelles has been evaluated at constant drug concentration and increasing surfactant concentration, and the values of absorbance of monomer band have been used to calculate the binding constant by non-linear regression (full lines in Figure 4a) assuming a 1 : 1 interaction between the drug and the surfactant micelle, using equation (1).[38] The results are presented in Table 2.

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

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

The Gibbs free energy of binding of mitoxantrone to surfactant micelles can be obtained by the following equation:

$$\Delta G_b^0 = -RT \ln K_b \quad (2)$$

where R is the gas constant and T the absolute temperature. The results are presented in Table 2.

Analysis of the data in Table 2 indicates that the binding constants of mitoxantrone monomers to Triton X-100, Tween 20 and Tween 80 micelles are about one order of magnitude higher at pH 10 than at pH 7.4. For neutral surfactant micelles, the binding is expected to be dominated by hydrophobic interactions. As a consequence, at pH 10, when the drug is non-protonated (charged), the values obtained for the binding constants for all three surfactants are higher than the values obtained at pH 7.4 when the drug exits as dication. On changing the surfactant, at pH 7.4 and pH 10, the values of binding constants follow the order Triton X-100 < Tween 20 < Tween 80. The Tween surfactants are mainly polyoxyethylene sorbitan combined with alkyl chains of different fatty acids: C_{12}–laurate (Tween 20) and C_{18}–oleate (Tween 80). It can be observed that the larger non-polar tail in Tweens induces a stronger complex formation at pH 7.4 and pH 10, indicating a correlation of binding with hydrophobicity.

The differences in binding constants between Tweens and Triton X-100 mainly come from the environmental specificities of micelles. There is a relation between micellar local polarity and polyoxyethylene residues of non-ionic surfactants. Lower polarity leads to higher binding constant.[2,39] The number of polyoxyethylene groups in Tween surfactants is 20 while in Triton X-100 it is on average 9.5. The micellar local polarity is therefore lower in Tween 20 and Tween 80 micelles than in Triton X-100 micelles. The micellar local polarity decreases, whereas mitoxantrone–non-ionic surfactant micelle binding constants increase with increasing polyoxyethylene content.

The binding constants for the interaction of mitoxantrone monomers with non-ionic surfactant micelles are much lower than the binding constants for the SDS and CTAB micelles.[22,23]

Drug–micelle interaction can be evaluated by the binding constant (K) and the partition coefficient (K_x), a thermodynamic parameter that represents the affinity of a given solubilizate to the micellar phase, relative the aqueous one. The partition coefficient depends on the structure of the drug and of the surfactant the micelles. The partition coefficient is important not only in elucidating the mechanism of solubilization but also in understanding biological phenomena such as the interaction between drugs and biological membranes. According to the pseudo-phase model,[40,41] the partition coefficient can be determined from the following equation:

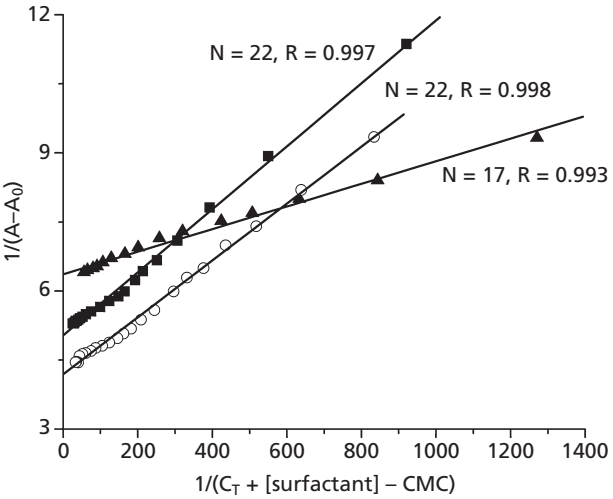

Figure 5 Plot of $1/(A - A_0)$ versus $1/(C_T + [\text{surfactant}] - \text{CMC})$ (equation 3) for mitoxantrone in Triton X-100 (▲), Tween 80 (o) and Tween 20 (■) solutions at pH 10 (N is the number of experimental points and R is the correlation coefficient).

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

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 $1/(C_T + [\text{surfactant}] - \text{CMC})$, as shown in Figure 5.

From equation (4), the standard free energy change for the transfer of mitoxantrone from bulk aqueous phase to micellar phase is obtained. The results are summarized in Table 2.

$$\Delta G_x^0 = -RT \ln K_x \quad (4)$$

By comparing the partition coefficients obtained for the distribution of mitoxantrone molecules between water and micellar phases it can be observed that the values of K_x at pH 7.4 and pH 10 follow the order Triton X-100 > Tween 80 > Tween 20. The higher micellar partition coefficient of Triton X-100 than Tweens at both pH values can be related to the higher aggregation number of Triton X-100 (140)[42] than Tweens (around 60),[43,44] which is responsible for the greater micellar size of Triton X-100, which in turn helps to accommodate more drug molecules per micelle. The higher micellar partition coefficient of Tween 80 than Tween 20 can be explained on the basis of their hydrophobic chain length:

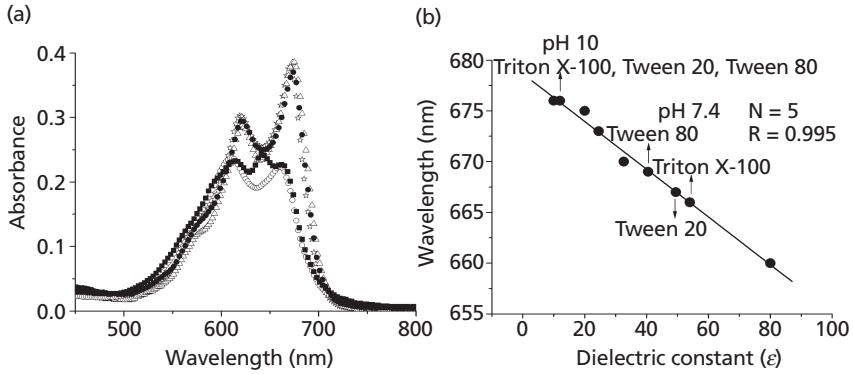

Figure 6 (a) Visible absorption spectra of mitoxantrone in different solvents: phosphate buffer pH 7.4 (o), 1,4-dioxane (■), methanol (*), ethanol (●), propanol (Δ). (b) Absorption maxima of monomer (λ_M) in different solvents (water, methanol, ethanol, propanol, tertbutanol) as a function of the dielectric constants. Respective positions for the surfactants at pH 7.4 and 10 are shown in the graph.

C_{18}-oleate (Tween 80) and C_{12}-laurate (Tween 20). Being lipophilic in nature,[45] the drug would have higher affinity for a longer chain with higher hydrophobicity and hence higher micellar partition coefficient. The results show that the uncharged mitoxantrone molecule (pH 10) exhibits a larger partition coefficient than positively charged mitoxantrone (pH 7.4). These findings suggest that at basic pH the deprotonated mitoxantrone is incorporated more efficiently into the hydrophobic medium of non-ionic micelles, compared to physiological pH, when the protonated drug is predominant.

In non-ionic micellar systems, the drug may reside in three different environments: the hydrophobic core composed of the hydrocarbon tails of the surfactant molecules, the polyoxyethylene shell (palisade layer) that surrounds the core and the surface of the micelles.[46] As seen in Table 1, the visible spectra of mitoxantrone are red shifted in the presence of micellar concentrations of Triton X-100, Tween 20 and Tween 80 compared with those in phosphate buffer pH 7.4, the red shift increasing with surfactant in the order Tween 80 > Tween 20 > Triton X-100. This red shift indicates that mitoxantrone molecules are transferred from a highly polar phase (phosphate buffer) to a less polar site in micellar medium. 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,[45] therefore it prefers to move from polar aqueous medium in more hydrophobic medium like micelles.

In order to gain further insight about the localization of the mitoxantrone molecule in non-ionic surfactant micelles, the absorption spectra of the drug in micellar solutions were compared with those recorded in different solvents with decreasing polarities. The spectra in Figure 6a indicate that the reduction of the solvent polarity involved a red shift of both absorption maxima and that the relationship between the position of these maxima and the dielectric constant was linear (Figure 6b). As spectral shifts are generally interpreted as polarity changes of the immediate microenvironment of the drug molecule, the substitution of corresponding absorption maxima of monomer band for Triton X-100, Tween 20 and Tween 80 micelles at pH 7.4 allowed us to determine polarity values corresponding to effective dielectric constants of 54, 49.5 and 40.5, respectively. The hydrocarbon core of any micelle has a dielectric constant of 2–5,[47] quite similar to that of 1,4-dioxane. The spectra in Figure 2 show the splitting of the monomer band into two components in the presence of non-ionic micelles, which was not observed for SDS and CTAB micelles, but is more evident in the drug spectrum in 1,4-dioxane in Figure 6a. This behaviour allows assignment of the band around 647 nm, increasing with surfactant concentration, to the ionic–hydrophobic interactions of a part of the drug molecule present in a more hydrophobic medium. As the palisade layer composed of the polyoxyethylene chains has a dielectric constant of 40–50,[48] the major part (band at 660 nm) of the drug is most probably located in the palisade layer, which is known to be much thicker (25 Å) than the Stern layer of ionic micelles (6–9 Å),[49] similar to another antitumour antibiotic, epirubicin.[2] The position of the mitoxantrone molecule in non-ionic micelles shifts towards the more polar surface region of the micelles with a decrease in the number of carbon atoms in the hydrophobic tail of the surfactant molecules. At pH 10, the red shift is similar for all non-ionic micelles (16 nm) and the dielectric constant determined in Figure 6b is about 12, indicating that uncharged mitoxantrone molecules penetrate deeper into non-ionic micelles.

Conclusions

The present paper reports the spectral results regarding the interaction of mitoxantrone with non-ionic surfactants (Triton X-100, Tween 80 and Tween 20) in submicellar and micellar surfactant concentrations at pH 7.4 and pH 10. In

the submicellar surfactant concentration range no interaction between mitoxantrone and all non-ionic surfactants was observed. Above the CMC, spectral data showed that the micellization reduces the aggregation of the drug and mitoxantrone is incorporated in micelles in the monomer form. The changes in the absorption spectra at micellar surfactant concentrations and the enhancement of absorbance were rationalized in terms of binding constants, Gibbs free energy of binding, partition coefficients and the standard free energy change for the transfer of mitoxantrone from bulk water to micellar phase. The strength of the interaction between mitoxantrone and non-ionic micelles is higher at pH 10 than at pH 7.4, and depends on the surfactant in the order Tween 80 > Tween 20 > Triton X-100. The partition coefficients obtained for the distribution of mitoxantrone molecules between water and micellar phases are higher at pH 10 than at pH 7.4 and follow the order Triton X-100 > Tween 80 > Tween 20. Regarding the position of mitoxantrone molecule in non-ionic micelles, the spectral results indicate that the larger part of the drug is most probably located in the palisade layer and a small part resides in a more hydrophobic medium.

The results for simple model systems miming the drug–membrane interactions represent a first step towards understanding the action and biological properties of anticancer drugs, as the cell membrane is the first barrier encountered by these drugs, and help to elucidate the behaviour of the drug *in vivo*, as well the possible utilization of surfactant micelles as drug carriers.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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