Scientific paper

Insight Into the Interaction of Quinizarin with SDS Micelles – Effects of Additives

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Abstract

Association behavior between quinizarin (1,4-dihydroxy-9,10-anthraquinone, Q), an analogue of the chromophore of anthracycline anticancer drugs and sodium dodecyl sulfate (SDS) micelles in the presence of glucose, NaCl and urea additives was studied using absorption spectroscopy and conductometric techniques. The spectral results indicate an increase of binding constant and partition coefficient values in the presence of glucose and NaCl whereas the addition of urea leads to a decrease of binding strength and quinizarin partitioning into SDS micelles. Thus, the rise of NaCl and glucose concentrations is favorable for the quinizarin distribution into SDS micelles. From electrical conductivity measurements it was found that the critical micelle concentration (CMC) of SDS/quinizarin system decreases by adding NaCl and glucose whereas urea has not influence on the micelization process at the concentrations used in the present study. Since biologically compounds like glucose, NaCl and urea are found in the human body, the attained outcomes can be important in finding of effective drug delivery systems.

Keywords: Quinizarin, SDS micelles, glucose, NaCl, urea

1. Introduction

Quinizarin (1,4-dihydroxy-9,10-anthraquinone, Q) is a synthetic anthraquinone used as fungicide and pesticide, antioxidant, additive in lubricants to check oxidation and corrosion in engines, and as dye.[1,2] Quinizarin inhibits HIV proteinase[3] and possess significant antiproliferative and antimetastatic properties by the induction of intracellular transglutaminase activity.[4,5] From pharmaceutical point of view, quinizarin molecule contains the planar anthraquinone unit typical of some biologically and pharmaceutically significant compounds, including several antitumor drugs such as doxorubicin, daunorubicin and mitoxantrone which are widely used in clinical practice. The anthraquinone chromophore is responsible both for antitumor activity of these drugs by the intercalation between the DNA base pairs and cardiac toxicity by the generation of reactive oxygen species. Taking into account the above, different simpler and cheaper anthraquinones (quinizarin, danthron, purpurin) have been investigated and compared with the known drugs.[6–9]

Surfactants with their unique structure that contains both hydrophilic and hydrophobic moieties in the same molecule can form aggregates (micelles) at a certain concentration (known as critical micelle concentration, CMC) due to a delicate balance between the interactions of polar and non-polar parts. Besides their extensively use in the textile and pharmaceutical industries as solubility enhancers, diluents or emulsifying agents, surfactant micelles can be used in drug delivery systems to increase the solubility and bioavailability of hydrophobic drugs and to protect the drug molecules from adverse effects of the biological environment.[10–13] The solubilization of drug molecules into micelles depends on its polarity: nonpolar molecules will be solubilized in the micellar core while the drug molecules with intermediate polarity will be distributed in the interfacial region of the micelle in certain intermediate positions.[14–16]

The development of an effective drug delivery system demands in-depth knowledge of the interaction of drug molecules with surfactant micelles and the effect of several factors like pH, temperature, additives, ionic strength, etc.[17,18] Due to their structure, the surfactant micelles mimic the native lipid bilayer environment and are used to study the interactions of different drugs with membranes.[19,20]

Sodium ions, glucose and urea are present in blood plasma in variable quantities and their presence may influence the drug biological activity. In addition, the administration of anthracycline drugs is given as slow infusion in 0.9% NaCl or 5% glucose solutions and glucose is used as a preferred source of carbohydrate in parenteral nutrition regimens, being rapidly absorbed from the gastrointestinal tract. It is well documented that the addition of various additives like electrolytes, carbohydrates, alcohols, aminoacids, etc. can affect the association behavior of ionic and nonionic surfactants either through specific interactions with the surfactant molecules or by changing the solvent nature.[21–24] The presence of these compounds not only changes the micellar parameters but also can modulate the interaction pattern of micelles with drugs. Hence, it is important to get knowledge of drug–micelle association behavior in the presence of different physiological additives.

We previous investigated the interaction of quinizarin with SDS micelles in 0.1 M phosphate buffer (pH 7.4) and at different temperatures, using spectrophotometric and conductometric techniques.[25] The results showed a strong interaction between quinizarin and SDS micelles and both binding and partition processes are spontaneous and entropy driven. Also the hydrophobic interactions are the main forces involved in binding and partition processes.

Considering the above aspects and in extension to our prior study concerning the interaction of antitumor drugs with biomimicking organized assemblies like surfactant micelles, the current paper aims to investigate the influence of glucose, NaCl and urea on the interaction of quinizarin with SDS micelles. The studies were carried out using absorption and electrical conductance measurements.

2. Experimental

2. 1. Materials

Quinizarin (96% purity), sodium dodecyl sulfate (SDS, 97%), sodium chloride (NaCl, 99%), glucose (99.5%), urea (99%), sodium phosphate dibasic (99%) and sodium phosphate monobasic (99%) were purchased from Sigma Aldrich and used as received without further purification. All solutions were prepared using deionized water of 18.2 MΩcm resistivity (Direct-Q 3UV System, Millipore). Experiments were performed in 0.1 M phosphate buffer, pH 7.4 in order to mimic physiological conditions. Due to the low solubility of quinizarin in water, a concentrated stock solution was prepared by dissolving quinizarin in methanol. Then, small aliquots of this stock were diluted with phosphate buffer such that the methanol content in the investigated solutions was always below 1%. The solutions were prepared just before experiment and kept in the dark because the quinone unit is sensitive to the light.

2. 2. UV-Visible Measurements

Absorption spectra were taken on a JASCO V-630 spectrophotometer equipped with a Peltier controlled ETCR-762 model accessory (JASCO Corporation, Tokyo, Japan) using quartz cuvette with a path length of 1 cm. The absorption spectra of quinizarin in 0.1 M phosphate buffer, pH 7.4 and different concentrations of glucose, NaCl and urea have been recorded in the range of wavelength λ = 350–700 nm after the successive additions of concentrated SDS solution.

2. 3. Conductivity Measurements

Specific conductivities were performed on Consort K912 conductivity meter (Parklaan 36, B-2300 Turnhout, Belgium). This instrument has auto ranging from 0 to 1000 mS/cm and conductivity control with accuracy of ± 0.5%. The electrode used had a cell constant of 0.98 cm^{-1} and was calibrated using KCl over the appropriate concentration range. A concentrated SDS solution was gradually added to phosphate buffer containing quinizarin and different concentrations of additives and the conductivity of the ensuing solution was noted, after appropriate mixing. Subsequently, CMC values were determined by using the conventional method based on the plot of conductivity (k) against the surfactant concentration.

3. Results and Discussion

3. 1. Absorption Spectroscopy

Our previous spectral investigation on the interaction of quinizarin with SDS micelles in 0.1 M phosphate buffer (pH 7.4) at different temperatures revealed a strong interaction between quinizarin and SDS micelles.[25] In order to find out the effect of physiologic additives such as glucose, NaCl and urea on the interaction of quinizarin with SDS micelles, similar experiments were performed in the presence of 1%, 5% glucose, 0.5%, 0.9% NaCl and 0.6%, 1.2% urea.

Fig. 1 shows the absorption spectra of quinizarin in the presence of additives (5% glucose (Fig. 1a) and 0.5% NaCl (Fig. 1b)) and increasing concentrations of SDS. In our experimental conditions (0.1 M phosphate buffer, pH 7.4), quinizarin is in neutral form and the visible absorption spectrum shows a broad absorption maximum at ~ 470 nm and a shoulder at about 535 nm. The changes in the spectral behaviour of quinizarin for increasing SDS concentrations are the same for glucose, NaCl and urea, respectively the increase in absorbance and the splitting of the absorption maximum in three peaks. Also, a new peak appears around 515 nm and the shoulder at about 535 nm disappears. Addition of glucose, NaCl and urea does not affect the characteristics of absorption spectra of quinizarin in SDS micelles, which would imply the presence of the same kind of interactions and the same location of

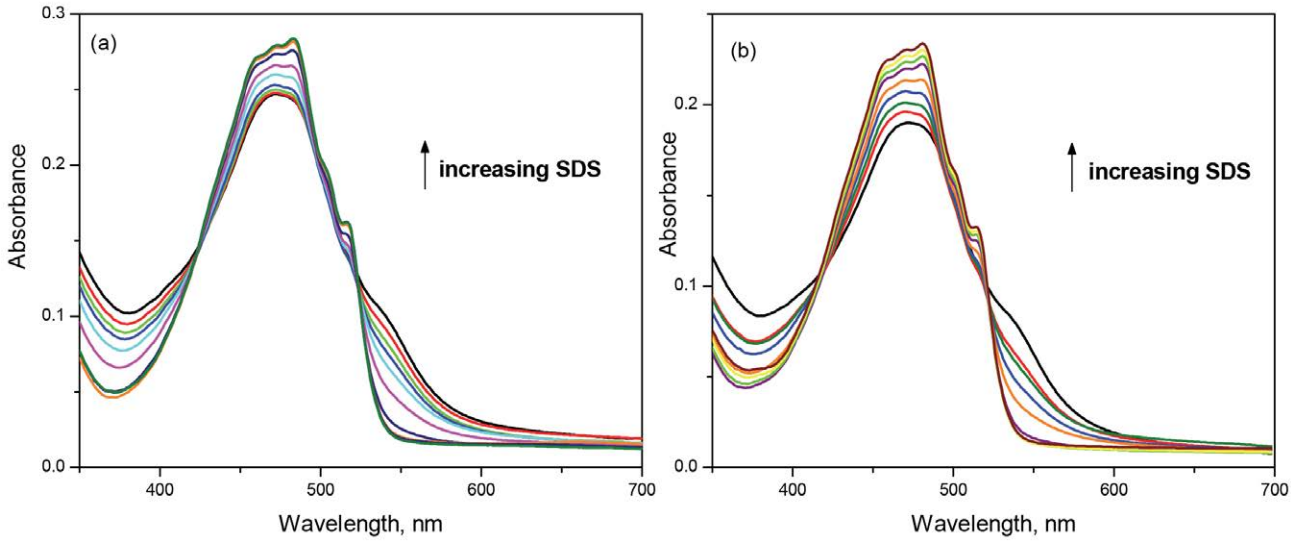

Figure 1. Visible absorption spectra of quinizarin at various concentrations of SDS in the presence of: (a) 5% glucose and (b) 0.5% NaCl.

quinizarin molecules into SDS micelles as compared with the absence of these additives.

Further, the absorbance values at 470 nm were used to calculate the binding constant (K_b) and the partition coefficient (K_x), and the respective thermodynamic parameters in order to evaluate the influence of glucose, NaCl and urea additives on the interaction of quinizarin – SDS micelles. The binding constant was determined using the Benesi–Hildebrand equation:[26,27]

$$\frac{1}{A - A_0} = \frac{1}{K_b (A_1 - A_0)[SDS]} + \frac{1}{A_1 - A_0} \quad (1)$$

where, A_0 is the absorbance value in the absence of SDS, A is the absorbance value in the presence of SDS and A_1 is the absorbance value at high concentration of SDS. From the linear plots between $1/(A–A_0)$ and 1/[SDS] (Fig. 2), the values of K_b were evaluated using the intercept and the slope and are given in Table 1.

The binding constant of quinizarin to SDS micelles was previous found to be 2524 M^{-1}.[25] From Table 1 it can be observed that glucose and NaCl enhance the binding of quinizarin to SDS micelles and this increase is higher for higher concentrations of glucose and NaCl. The high-

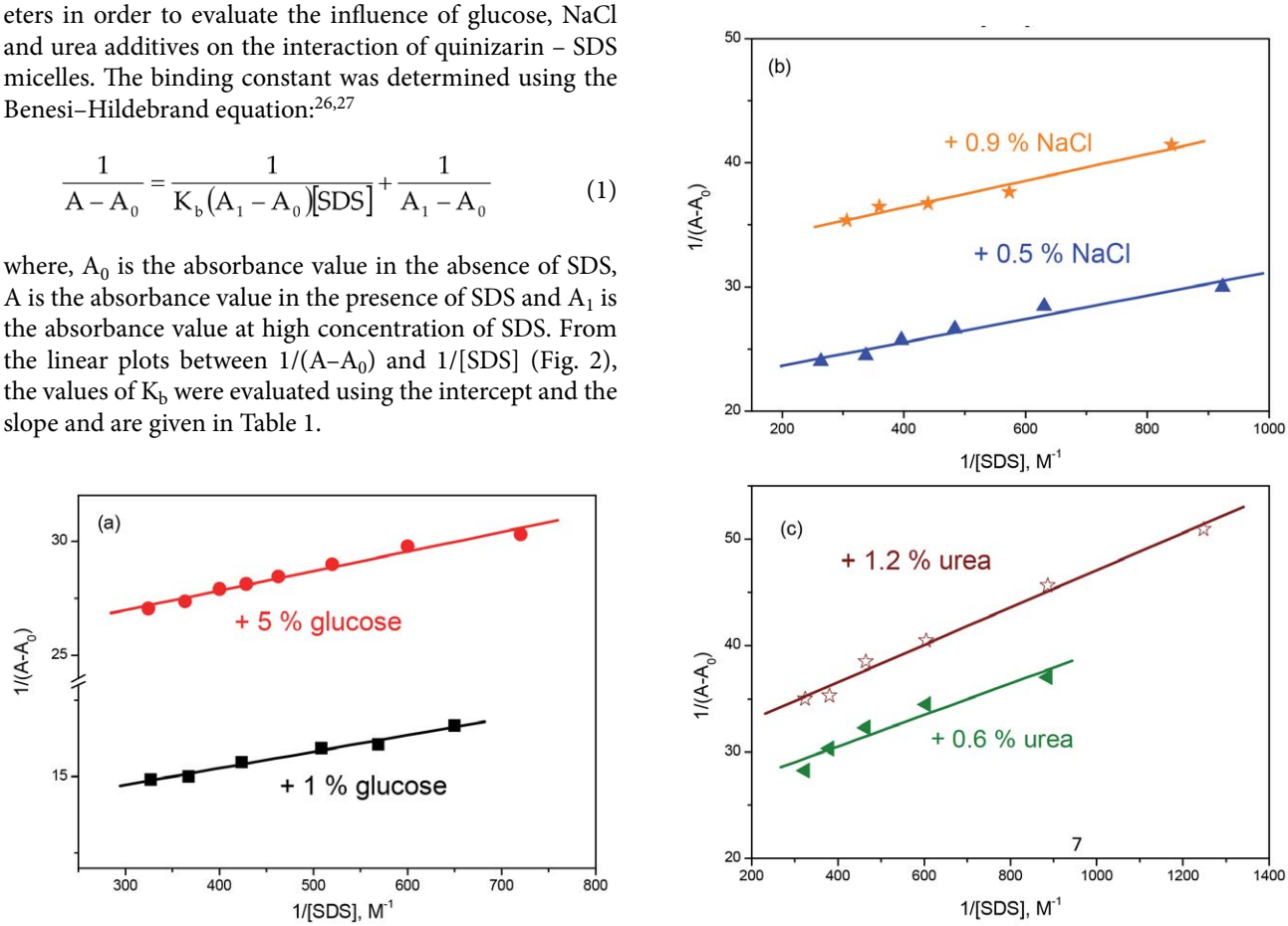

Figure 2. Benesi–Hildebrand plots for the calculations of binding constant (K_b) for quinizarin/SDS micellar system in the presence of additives: a) glucose, b) NaCl and c) urea.

est binding constant was obtained for 0.9% NaCl. Banipal *et al.* reported that the presence of NaCl increases the binding and partitioning of ciprofloxacin hydrochloride to SDS and hexadecyltrimethylammonium bromide (HTAB) micelles because NaCl enhances the hydrophobic interactions between the drug and both surfactants.[28] We have reported that the hydrophobic interactions are the main forces involved in the binding of quinizarin to SDS micelles.[25] Therefore, the increase of hydrophobic interactions can account for higher binding constants observed for quinizarin/SDS micelles in the presence of NaCl.

An increase of the interaction strength in the presence of glucose, galactose, sucrose and maltose carbohydrates was found for the binding of safranine T dye with different micelles.[20] Glucose is a hydrophilic molecule containing six hydroxyl groups that appears to be responsible for making strong cooperative hydrogen bonds with the surfactant molecules which is manifested by dominance of electrostatic interactions at pre-micellar SDS concentrations whereas hydrophobic interactions play a greater role at micellar SDS concentrations.[29]

Unlike glucose and NaCl, the presence of urea leads to a decrease of the binding constant of quinizarin to SDS micelles. The decrease of the binding constants on addition of urea was also observed in the case of the binding of the charged and uncharged forms of the local anesthetic tetracaine to zwitterionic micelles.[30] Electron spin resonance spectroscopy investigation on the effect of urea on SDS micelles showed a decrease of the polarity and a strong increase of the microviscosity of the micellar interface.[31]

The negative values of ΔG_b indicate the spontaneous nature of quinizarin-SDS micelles binding process in the presence of glucose, NaCl and urea additives and the spontaneity of this process is enhanced in the presence of higher glucose and NaCl concentrations.

Along with the determination of binding constant, the quinizarin–SDS micelles interaction was further characterized by determining the partition coefficient (K_x) which is a thermodynamic parameter representing the ratio of concentration of drug molecules in micelle to that in aqueous solution and provides information about the extent of solubilization. The partition coefficient was determined according to the pseudo-phase model using the following equation:[32,33]

$$\frac{1}{\Delta A} = \frac{1}{\Delta A_\infty} + \frac{n_W}{K_x \Delta A_\infty ([SDS] + C_T - CMC)} \quad (2)$$

In eq. (2), $\Delta A = A - A_0$, $\Delta A\infty = A_1 - A_0$, C_T is the total drug concentration and $n_w = 55.5$ M is the molarity of water. The values of K_x are obtained from the slope of the plot of $1/\Delta A$ versus $1/([SDS] + C_T - CMC)$, as is shown in Fig. S1 (Supplementary Material).

Our previous investigation showed that quinizarin presents large positive values of K_x (3.44×10^5) indicating that quinizarin molecules prefer to move from aqueous environment to more hydrophobic environment of SDS micelles.[25] As seen from Table 1, NaCl, glucose and urea exert a strong influence on the degree of partitioning of quinizarin molecules. The presence of NaCl and glucose leads to higher partition coefficients, meaning large-scale transfer of quinizarin molecules from the aqueous to the micellar media. The K_x values decrease in the presence of urea. The large negative values of ΔG_x are indicative of the spontaneous nature of partitioning process in the presence of these additives.

The radius of SDS micelles, the aggregation number and the packing parameter increase with increasing NaCl concentration.[21] This means that larger spherical micelles are formed which in turn helps to accommodate more drug molecules per micelle. Also, the aggregation number of SDS increase in the presence of different sugars (glucose, galactose, sucrose, maltose).[22] Therefore, the higher micellar partition coefficients achieved in the presence of increasing concentrations of glucose and NaCl can be related to the greater micellar size of SDS micelles.

3. 2. Conductivity Measurements

The conductivity of quinizarin/SDS solutions as a function of surfactant concentration is viewed in Fig. 3 for different concentrations of glucose, NaCl, and urea. From the sudden change of the slope in these plots the CMC values were obtained and included in Table 2.

The CMC of SDS in pure water at 298.15 K is between 8.00 and 8.30×10^{-3} M depending on the exper-

Table 1. Values of binding constant (K_b), partition coefficient (K_x) and free energy of binding (ΔG_b^0) and partition (ΔG_x^0) for quinizarin/SDS micellar system in 0.1 M phosphate buffer (pH 7.4) and different additives.

Additive	K_b / 10^3 (M^{-1})	ΔG_b^0 (kJ mol^{-1})	K_x / 10^5	ΔG_x^0 (kJ mol^{-1})
glucose 1%	2.62 ± 0.05	–19.17	5.11 ± 0.03	–32.02
glucose 5%	2.83 ± 0. 07	–19.36	6.70 ± 0.05	–32.68
NaCl 0.5%	2.53 ± 0.02	–19.09	4.40 ± 0.04	–31.66
NaCl 0.9%	2.97 ± 0.03	–19.48	6.98 ± 0.07	–32.78
urea 0.6%	1.76 ± 0.02	–18.20	1.13 ± 0.05	–28.34
urea 1.2%	1.57 ± 0.04	–17.93	1.42 ± 0.04	–28.90

imental method used.[34–36] In phosphate buffer at pH 7 and 298.15 K, CMC values of SDS decrease from 6.09 × 10^{-3} M (5 mM phosphate buffer) to 1.99 × 10^{-3} M (50 mM phosphate buffer).[37] In our previous investigations, it was found that the presence of quinizarin increases the CMC of SDS in 0.1 M phosphate buffer, pH 7.4 from 9.28 × 10^{-4} M to 1.06 × 10^{-3} M and this increase was explained by the possibility of hydrogen bonding between hydrophilic parts of drug and water, as the location of drug molecules in the outer portion of micelle close to micelle water interface leads to decrease in entropy thus making process of micellization less convenient.[25,38,39]

The results in Table 2 indicate that the presence of glucose leads to a decrease of CMC and increasing the concentration of glucose from 1% to 5% increases the reduction in CMC. These results are in agreement with literature results which indicate a decrease of CMC of SDS with gradually increasing concentrations of different sugars (glucose, galactose, sucrose, maltose).[22] Also, CMC values of SDS showed a regular decrease with increase in sugar concentration as well as with the size of the hydrophobic group of sugar molecule.[40] Glucose is a hydrophilic molecule containing six hydroxyl groups which strongly attract water molecules, thus the water – water interaction is replaced by water – sugar interaction and therefore the formation of iceberg structure around surfactant monomers due to hydrophobic interaction is prohibited and the micelle formation is favoured and CMC is lowered.[22]

A more pronounced decrease in CMC was observed by adding NaCl at SDS solutions containing quinizarin drug (Table 2). This indicates that higher concentration of NaCl provides a convenient environment for micellization of our studied quinizarin/SDS system. In the case of ionic surfactants such as SDS, a decrease of CMC values was observed for the micellization of pure ionic surfactants and also for drug-surfactant systems as the inorganic salt concentration increases.[18,21,41–43] The decrease in the CMC value is mainly due to the decrease in the thickness and potential of the electric double layer at the interface, and consequently, the electrical repulsion between charged head groups are reduced and the micellization process starts at lower surfactant concentration.[41–43]

Regarding the influence of urea, it can be observed that the presence of 0.6% and 1.2% urea does not change the CMC value of quinizarin/SDS solution. Reports on urea effects on SDS micellization in aqueous solution indicate that the CMC increased upon 2, 4 or 6 M urea concentrations addition, whereas the micellar aggregation number and the polarity the micellar interface decreased.[44] The lower urea concentrations used in our investigations has not influence on the micellization of quinizarin/SDS system, in agreement with studies performed by Kancharla et al. which showed that the CMC of SDS in aqueous solution did not change much at low urea concentrations, but increased by 11% in the presence of 4 M urea.[45]

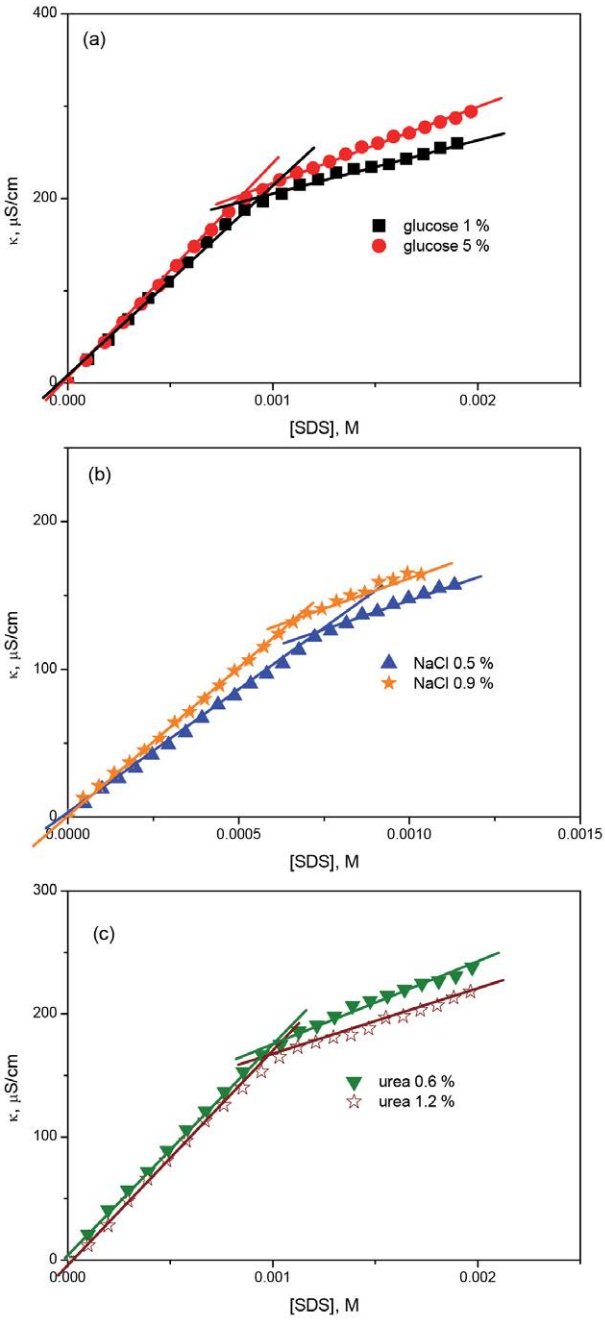

Figure 3. Dependence of specific conductivity, κ, on the concentration of SDS in solution of 2.15 × 10^{-5} M quinizarin in 0.1 M phosphate buffer in the presence of different concentrations of (a) glucose, (b) NaCl and (c) urea.

4. Conclusions

The present study focuses on the effects of glucose, NaCl and urea additives on the interaction between quinizarin, an analogue of the chromophore of anthracycline anticancer drugs and SDS micelles, as a model drug delivery system and the most accepted model system for studying different aspects of membrane interactions with

Table 2. Critical micelle concentration (CMC) for SDS in: water, 0.1 M phosphate buffer (pH 7.4), 0.1 M phosphate buffer (pH 7.4) and 2.15×10^{-5} M quinizarin, and 0.1 M phosphate buffer (pH 7.4), 2.15×10^{-5} M quinizarin and different additives (NaCl, glucose, urea).

Medium	CMC, M
water[36]	8.00
0.1 M phosphate buffer (pH 7.4)[25]	$(9.28 \pm 0.11) \times 10^{-4}$
0.1 M phosphate buffer (pH 7.4)[25] + quinizarin	$(1.06 \pm 0.08) \times 10^{-3}$
glucose 1%	$(9.32 \pm 0.09) \times 10^{-4}$
glucose 5%	$(8.95 \pm 0.08) \times 10^{-4}$
NaCl 0.5%	$(7.52 \pm 0.10) \times 10^{-4}$
NaCl 0.9%	$(7.02 \pm 0.08) \times 10^{-4}$
urea 0.6%	$(1.02 \pm 0.09) \times 10^{-3}$
urea 1.2%	$(9.93 \pm 0.02) \times 10^{-4}$

drug molecules. These additives are found in the human body and their presence may affect the micellization tendencies of surfactants. The spectral results show that the presence of glucose, NaCl and urea has a pronounced influence on the binding and partitioning process: glucose and NaCl enhance the quinizarin binding and distribution ability into SDS micelles, whereas the addition of urea has an opposite effect. The obtained CMC values of quinizarin/SDS system were found to undergo a decrease in presence of NaCl and glucose, whereas urea has not influence on the micelization process at the concentrations used in the present study.

The results of the present study may provide valuable information in seeking better drug formulation and drug delivery systems taking into account that glucose, NaCl and urea are present in body fluids.

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Povzetek

Z absorbcijsko spektroskopijo in konduktometričnimi tehnikami smo proučevali tvorbo asociatov med kinizarinom (1,4-dihidroksi-9,10-antrakinonom, Q), analogom kromofora antraciklinskih protirakavih zdravil, in miceli natrijevega dodecil sulfata (SDS) v prisotnosti dodatkov glukoze, NaCl in sečnine. Spektralni rezultati kažejo na povečanje vrednosti vezavne konstante in porazdelitvenega koeficienta v prisotnosti glukoze in NaCl, medtem ko se ob dodatku sečnine vezavna moč in vgrajevanje kinizarina v SDS micelle zmanjša. Povečanje koncentracij NaCl in glukoze je torej ugodno za vgraditev kinizarina v SDS micele. Iz meritev električne prevodnosti smo ugotovili, da se kritična micelna koncentracija (CMC) sistema SDS/kinizarin zmanjša z dodajanjem NaCl in glukoze, medtem ko sečnina nima vpliva na proces micelizacije pri pogojih, uporabljenih v tej študiji. Ker so biološke spojine, kot so glukoza, NaCl in sečnina, prisotne v človeškem telesu, imajo izsledki študije potencialno uporabo pri razvoju učinkovitih sistemov za dostavo zdravil.

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