INTERACTION AND MICELLAR SOLUBILIZATION OF DICLOFENAC WITH CETYLTRIMETHYLAMMONIUM BROMIDE: A SPECTROPHOTOMETRIC STUDY

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Received February 17, 2008
Accepted October 1, 2008
Published online March 17, 2009

In this study, the interaction of diclofenac (Dic) with cationic surfactant cetyltrimethylammonium bromide (CTAB) was investigated. The effect of cationic micelles on solubilization of diclofenac in aqueous micellar solution was studied at pH 6.8, 29 °C and various drug concentrations. The binding of diclofenac to CTAB micelles was accompanied by a batochromic shift in the drug absorption spectra. The solubility of diclofenac increased with increasing surfactant concentration as a consequence of the association between the drug and micelles. From the results, the binding constants K_b, was obtained. By using the pseudo-phase model, the partition coefficient between the bulk water and CTAB micelles, K_x, and the Gibbs energy of binding were calculated. The value of binding constant and partition coefficient are increased by increasing of diclofenac concentration.

Keywords: Sodium diclofenac; Cetyltrimethylammonium bromide; Micellar solubilization; Binding constant; Partition coefficient; Surfactants, Micelles.

Surfactants are amphiphilic substances the molecules of which consist of both hydrophilic and hydrophobic regions. These substances are known to play a vital role in many processes of interest in both fundamental and applied sciences[1,2]. Micelles are aggregates formed by surfactants above their critical micelle concentration (CMC). They are composed of hydrophilic surface and hydrophobic core. This specific structure makes the micelles capable of establishing chemical and physical interactions with either hydrophilic or lipophilic substances, which can be exploited in pharmaceutical analysis[3–5]. The micelles exhibit an interfacial region separating the polar bulk aqueous phase from the hydrocarbon-like interior[1]. As a consequence, micellar solutions consist of a special medium in which hydrophobic, amphiphilic or ionic compounds can be solubilized and concentrated or separated in aqueous solution[6]. Moreover, the position of a solubilized drug

Collect. Czech. Chem. Commun. 2009, Vol. 74, No. 3, pp. 503–513

doi:10.1135/cccc2008021

in a micelle depends on its polarity: nonpolar substances are solubilized in the micellar core whereas substances with intermediate polarity are distributed along with the surfactant molecules in certain intermediate positions.

Drug interactions with heterogeneous media (micelles, lipid bilayer vesicles, and biomembranes) induce changes in some physicochemical properties of the drugs (solubility, spectroscopic, acid base properties etc.)[7–11]. By monitoring these changes it is possible to quantify the degree of drug/micelle interaction which is normally expressed as the drug/micelle binding constant, K_b and micelle/water partition coefficient, K_x. The determination of these constants is important for the understanding of interactions with biomembranes as well as for the quantitative structure–activity relationships (QSAR) of drugs[12]. These values are also important if surfactants are used in HPLC or micellar electrokinetic capillary chromatography (MEKC) in drug quality control[13–17].

Most therapeutic drugs are selected or designed to be amphiphilic in order to penetrate cells and tissues and to favor interaction of drug molecules with receptor sites. Hence, study of drug interactions with surfactants and its distribution between the micelle and bulk water phases can be helpful in the understanding of drug distribution in the cell wall and cells and of its effect. Surfactants are also widely used in analytical determination of various drugs and the quality control of drugs in pharmaceuticals[19]. Sodium diclofenac (Dic), or sodium 2-[(2,6-dichlorophenyl)amino]phenyl acetate, is a non-steroidal anti-inflammatory drug (NSAID) which is widely used as a therapeutic agent for rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute gouty arthritis (Fig. 1b)[18]. Similarly to other NSAIDs, the diclofenac use is associated with rare, but serious and sometimes fatal, gastrointestinal (GI) side effects. Sodium diclofenac is an ideal candidate for

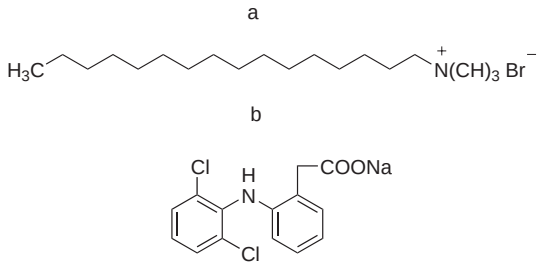

FIG. 1
Molecular structures of cetyltrimethylammonium bromide (CTAB) (a) and sodium diclofenac (Dic) (b)

formulations in the sustained release form due to its rapid elimination and its adverse gastrointestinal reaction. Work has been done to formulate ideal sustained-release tablets of sodium diclofenac using polymers. The literature shows numerous efforts of using surfactants in drug formulations. Surfactants sometimes act synergistically with a drug substance to promote its absorption or activity or may decrease its activity by entrapping the drug molecule in micelles, thus, helping in sustained release of the dose. To elaborate the actual behavior of drug in micellar media, detailed physicochemical studies are required[19,20].

In the here, the diclofenac drug has been selected for our study because of the wide applications of diclofenac. The interaction of a cationic surfactant, cetyltrimethylammonium bromide (CTAB; Fig. 1a) with diclofenac drug and its distribution between the micelle and water phases was investigated. This study can be helpful in understanding the diclofenac effect and distribution in cells due to similar micelles the cell wall. Absorption spectrophotometry was used to quantify the Dic/CTAB binding constant and micelle/water partition coefficient of drug by applying the mathematical models that consider partitioning of the drug between the micellar and aqueous pseudo-phases[5].

EXPERIMENTAL

Materials and Instruments

Cetyltrimethylammonium bromide (CTAB) was obtained from Sigma chemical Co. All the salts (Merck) were of the highest purity and used without further purification. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate was obtained from Scharlau Co., and used for buffer solution preparation. Pure sodium diclofenac powder was a gift from Drug Applied Research Center (Tabriz, Iran). All pH measurements were made at 29 °C, using Metrohm 744 (Switzerland). Conductometric measurements have been done using Jenway 470 (EU). Absorption spectra were recorded on a Perkin–Elmer Lambda 25, double-beam UV-Vis spectrophotometer with 1.0 cm quartz cuvettes at 29 °C. Phosphate buffer solutions were prepared and used for preparing all of the solutions. Double distilled water was used for preparation of buffer solutions.

Methods

pH and ionic strength (0.05 M) were adjusted using buffer solution, and the measurements were performed at room temperature (29 °C). Stock solutions of sodium diclofenac (1×10^{-2} mol dm^{-3}) were prepared by dissolving the compound in buffer solution. Stock solution of 1×10^{-1} mol dm^{-3} CTAB was prepared by dissolving an appropriate amount of the surfactant in buffer solution. Diclofenac solution (1 ml) was transferred to the spectrophotometer cuvette and small volumes of the CTAB stock solution were added. The varia-

tion of absorbance of diclofenac solution by increasing the CTAB concentration was recorded at 287 nm using buffer solution as a blank.

The drug/micelle binding constant and micelle/water partition coefficient were determined from the absorbances at λ = 287 nm of a series of solutions containing fixed concentrations of sodium diclofenac (C_{Dic} = 1 × 10^{-6}, 1 × 10^{-5} and 1 × 10^{-4} mol dm^{-3}) and increasing concentrations of CTAB. The conductivity of solutions containing various constant concentrations of diclofenac was measured at pH 6.8 and adjusted ionic strength (0.05 M) at 29 °C. The conductometer was calibrated using potassium chloride reference solution that supplied with the Jenway Co. The conductivity of the experiment solutions (at various constant concentration of diclofenac) against surfactant concentration showed a clear break that has been occurred in the critical aggregation concentration. The values of CACs are determined from these breaks and collected in Table I. The ionic strength adjusted at 0.05 M due to this fact that at high ionic strengths electrostatic interactions decreased. At low ionic strengths the linear region also decreased. Any other conductivity measurements have been designed for proofing the effect of the ionic strength and buffer solution concentration that is in good agreements with the spectrophotometric measurements and previous reports about ionic strength and buffer solution effect on electrostatic and hydrophobic interactions (Fig. 2)[21,22]. According to the fact that diclofenac is a poor water soluble drug, so it would be acceptable that at high ionic strengths the solubility of drug and ion dissociation coefficient of drug decreased and affected the electrostatic interaction of diclofenac and CTAB (see Fig. 2).

TABLE I
The values of binding constant, partition constants, related Gibbs energies and CAC in diclofenac–CTAB interactions at 29 °C

pH	C_{Dic} mol dm^{-3}	K_b	ΔG_b^o kJ mol^{-1}	K_x	ΔG_x^o kJ mol^{-1}	CAC mol dm^{-3} [a]
6.8	1 × 10^{-6}	1.93	–1.657	11.02	–6.026	9.99 × 10^{-4}
	1 × 10^{-5}	53.36	–9.986	63.35	–10.417	2.49 × 10^{-3}
	1 × 10^{-4}	127.87	–12.180	225.03	–13.599	3.48 × 10^{-3}
3	1 × 10^{-4}	–	–	–	–	–
9	1 × 10^{-4}	143.64	–12.472	–	–	–

[a] Conductometric measurements.

RESULTS AND DISCUSSIONS

Figure 3 shows the variation of absorbance difference versus CTAB concentration. As it can be seen from Fig. 3, absorbance difference by increasing the CTAB has been reached to the constant value and remained constant by further increasing of CTAB concentration so in the plot of $1/\Delta A$ vs $1/C_{CTAB}$ related points remains constant also that is due to the aggregation of diclofenac and surfactant molecules. According to this fact that Benesi–

Hildebrond equation is linear, so the plot has been plotted at the concentrations below CAC and the binding parameters obtained from this plot. As fallows from the diclofenac absorption spectra at pH 6.8, the addition of the CTAB (stock solution) shows a sufficiently significant bathochromic

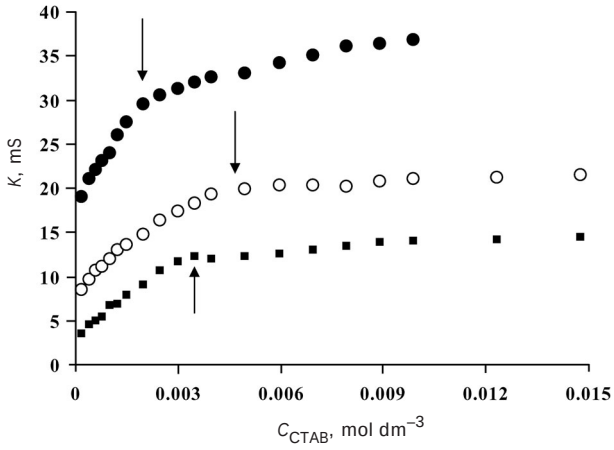

FIG. 2
The plot of conductivity versus CTAB concentration in 1×10^{-4} mol dm^{-3} diclofenac solution and various ionic strengths (in M): ● 0.5, ○ 0.05 and ■ 0.005

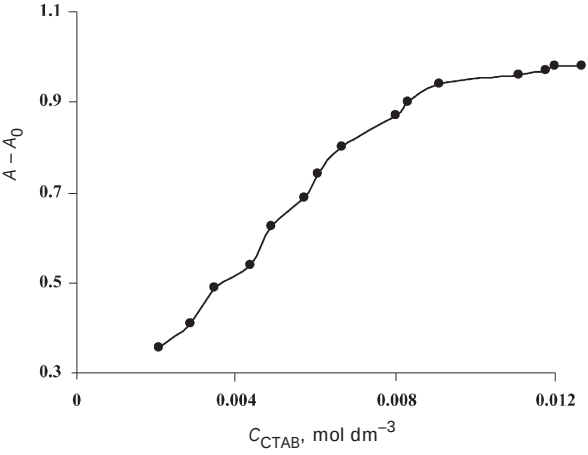

FIG. 3
The plot of $A - A_0$ vs CTAB concentration in 1×10^{-4} mol dm^{-3} diclofenac solution

shift of 11 nm of the higher wavelength maximum (Fig. 4). This shift indicates that diclofenac interacts with CTAB. It is well known that a solute can be arranged in the micelle in various ways. The position of a solubilized drug in a micelle depends on its polarity: non-polar molecules can be solubilized in the micellar core and substances of intermediate polarity are distributed along the surfactant molecules in certain intermediate positions[1,2]. The drug molecule may pass completely into the hydrophobic core or penetrate into the surface layer; it can be adsorbed on the surface of the micelle or, in the case of polar molecules it can be oriented with the polar part of the molecule in the surface layer and the non-polar part directed into the micelle. In order to solve the problem of a solubilized molecule position in the micelle it is useful to compare the spectra observed in the presence of the detergent with spectra in water and organic solvents of different polarities[4,5].

Determination of Binding Constant

The values of the binding constants K_b were obtained according to the method described previously[23–25]. By assuming that there is only one type of interaction between CTAB and diclofenac in aqueous solution, Eqs (*1*) and (*2*) can be established:

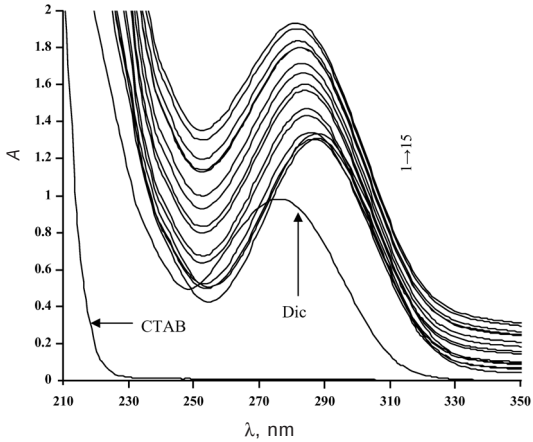

FIG. 4
Variation of 1×10^{-4} mol dm^{-3} diclofenac solution absorbance by increasing CTAB concentration (curves 1–15 increasing from 2 to 13 mmol dm^{-3}). The CTAB spectra have been recorded using pure 0.1 mol dm^{-3} CTAB solution

$$\text{Dic} + \text{Surfactant} \rightleftharpoons \text{Dic:Surfactant} \qquad (1)$$

$$K_b = \frac{[\text{Complex}]}{[\text{Dic}][\text{Surfactant}]} \qquad (2)$$

where K_b is binding constant, Eq. (*3*) can be derivate from absorbance measurements according to the manner that reported in the literatures[21,24,25]:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_{\text{Dic}}}{\varepsilon_b} + \frac{\varepsilon_{\text{Dic}}}{\varepsilon_b K} \frac{1}{C_{\text{Surfactant}}} \qquad (3)$$

where A_0 and A are the absorbances of diclofenac at 287 nm in the absence and presence of surfactant, respectively. ε_{Dic} and ε_b are the molar absorption coefficients of diclofenac and the complex, respectively. The plot of $1/(A - A_0)$ vs $1/C_{\text{Surfactant}}$ is linear and the binding constant (K_b) can be estimated from the ratio of the intercept to the slope[21,24,25].

Figure 5, shows the plot of $1/(A - A_0)$ vs $1/C_{\text{Surfactant}}$ at specified experimental conditions.

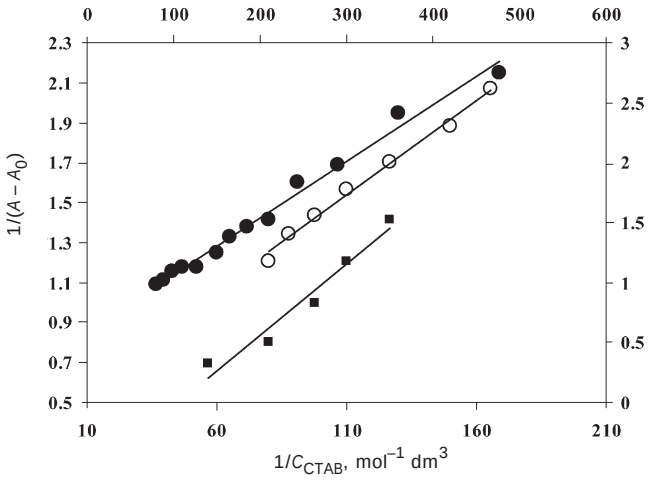

FIG. 5

The plot of $1/(A - A_0)$ vs $1/C_{\text{CTAB}}$ for diclofenac–CTAB interaction, where A_0 is the initial absorption band of pure diclofenac and A the recorded absorption at different CTAB concentrations; diclofenac concentration (in mol dm^{-3}): ● 1×10^{-4} (the scales for 1×10^{-4} mol dm^{-3} are shown in secondary X, Y axis), ○ 1×10^{-5} and ■ 1×10^{-6}

Determination of Partition Coefficient

The absorbance values obtained at λ = 287 nm can be also used for the calculation of partition coefficient K_x defined according to the pseudo-phase model as in[5]:

$$K_x = \frac{X_{Dic}^m}{X_{Dic}^w} \tag{4}$$

where X_{Dic}^m and X_{Dic}^w are the mole fractions of diclofenac in micellar and aqueous phase, respectively. They are related to the concentrations of the species in the solubilization system[5].

The fraction f can be calculated from the experimental data using Eq. (5):

$$f = \frac{\Delta A}{\Delta A^\infty} \tag{5}$$

where $\Delta A = A - A_w$ and $\Delta A^\infty = A^\infty - A_w$, A^∞ being the absorbance of diclofenac completely bound to the surfactant (CTAB).

Using Eqs (*4*) and (*5*), Eq. (*6*) can be written as below[5]:

$$\frac{1}{\Delta A} = \frac{1}{\Delta A^\infty} + \frac{1}{K_S \Delta A^\infty (C_{Dic} + C_{Surfactant} - CMC)} . \tag{6}$$

K_S and K_x ($K_S = K_x/n_w$) are obtained from the slope of the plot of $1/\Delta A$ vs $1/(C_{Dic} + C_{Surfactant} - CMC)$ as shown in Fig. 6.

The Gibbs energy of binding of diclofenac can be obtained from the following equations:

$$\Delta G_b^o = -RT \ln K_b . \tag{7}$$

From Eq. (*8*), the standard Gibbs energy change for the transfer of diclofenac from bulk water to the micellar phase can be obtained,

$$\Delta G_x^o = \mu_m^o - \mu_w^o = -RT \ln K_x . \tag{8}$$

Results are calculated and presented in Table I.

The effect of pH on diclofenac–surfactant interactions has been studied and the binding parameters has been calculated and collected in Table I. At

acidic pH the Benesi–Hildebrond linear behavior can not be seen in the plot of $1/(A - A_0)$ vs $1/C_{\text{Surfactant}}$ and so the K_b value and related standard Gibbs energy change can not be obtained. It is due to the fact that at low pH (pH 3 in this case) the diclofenac anions interacted with H^+ ions and the interaction between CTA^+ and diclofenac decreased strongly (pK_a(Dic) = 4.0)[26]. At pH 9 interaction of diclofenac with CTAB increased but by comparison with the neutral pH did not show significant difference. According to the fact that the studies in the neutral pH have a great interest in various studies, we select this pH for other studies.

It must be considered that in the presence of additives, surfactants make micelle like aggregate by these molecules that contribute in the aggregation phenomena. So we must call this point CAC (critical aggregation concentration). In the presence of additives, the CMC (or better CAC) of surfactant affected by the additive and depends on conditions that micellization has occurred. On the other hand, the CAC depends strongly on the experimental conditions. It is shown that the sodium diclofenac increased CMC or CAC of CTAB surfactant co-micelles[19]. On the other hand, diclofenac contributes to the micellization phenomena and increased the CAC that is necessary for aggregation. For proofing this point we design any conductometric measurements of CTAB CMCs in the presence of various concentrations of diclofenac and in the specified experimental conditions. The conductometric measurements shown that by increasing of diclofenac con-

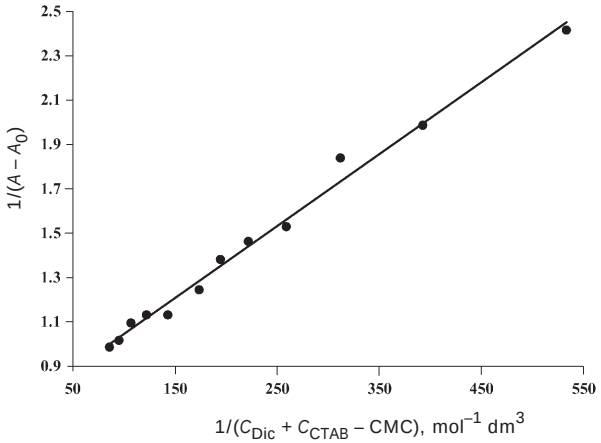

FIG. 6
Relation between $1/(A - A_0)$ and $1/(C_{\text{Dic}} + C_{\text{CTAB}} - \text{CMC})$ for 1×10^{-4} mol dm^{-3} diclofenac–CTAB interactions

centration the CAC values for CTAB-diclofenac aggregations increased, that is in agreement with other reports[19]. Results are collected in Table I. The partitioning phenomenon is depending on this co-micellization process and the drug partitioned between micelles and water phases.

The results show that the binding constant of the diclofenac interaction with CTAB increased by increasing diclofenac concentration. It is clear that the initial electrostatic interactions between ionic compounds (anionic diclofenac and cationic surfactant CTAB) are very important in these interactions but also the consequence hydrophobic interactions are important in binding processes. Judging from the diclofenac molecular structure, the hydrophobic interactions have a great role in diclofenac–CTAB molecular and micellar interactions. At low total concentrations of diclofenac and surfactant, head-to-head electrostatic interactions are the major interactions but at high concentrations due to increasing hydrophobicity, co-micellization proceeded of diclofenac, CTAB molecules and aggregates, and so hydrophobic interactions become important. The estimated binding Gibbs energy of diclofenac–CTAB interactions decreased consequently by increasing both diclofenac and CTAB concentrations, which is in good agreement with the co-micellization process as mentioned above (see Table I). From the results obtained for partitioning of diclofenac between the bulk water and CTAB micelles using pseudo-phase model, the partition constant, K_S and consequently K_x, was increased by increasing the diclofenac concentration, and consequently, the free energy of partitioning of diclofenac between the water and CTAB micelles decreased. So it can be concluded that diclofenac and CTAB molecules contributed to the micelle formation and that electrostatic and hydrophobic interactions show the key role in the co-micellization of diclofenac–CTAB.

This co-micellization that occurred between diclofenac and CTAB molecules can enhance the solubilization of diclofenac in the presences of CTAB micelles. The values of K_b and K_x are important if surfactants are used in HPLC or micellar electrokinetic capillary chromatography (MEKC) in diclofenac quality control.

Financial support of the Jahad-E-Daneshgahi of Urmia is gratefully appreciated.

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