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ORIGINAL ARTICLE

Study of inclusion complex formation between a cationic surfactant, two cyclodextrins and a drug

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Abstract In this study inclusion of hexadecyltrimethylammonium bromide (HTAB) with α-, and β-cyclodextrin (CD) in the presence and the absence of bromhexine (BH) was investigated using ion-selective electrode method. The association constants of HTAB with CDs were determined by potentiometry and were close to literature values. The obtained results indicated that α-CD formed 1:1 and 1:2 inclusion complexes, but β-CD formed only a 1:1 inclusion complex. In the presence of drug, the interaction between CDs and HTAB decreased, because both drug and HTAB could interact with CDs. The results showed that the interaction between drug and CDs are greater than HTAB and CDs. The stoichiometry of the inclusion complexes, the critical aggregation concentration (CAC), the monomer surfactant concentration of HTAB, $[HTAB]_f$, and also the effect of the inclusion complex on the micellization process of the HTAB were determined by conductivity measurements.

Keywords HTAB · Bromhexine · Cyclodextrin · Surfactant-selective electrode · Conductometry

Introduction

Cyclodextrins (CDs) (Fig. 1a) are macrocyclic oligosacharides, which consist of 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucopyranose units, linked by α-1, 4 bonds [1–3]. This circular configuration causes that the CDs become donut-shaped in solution with a non-polar interior and two polar rims [4]. The primary hydroxyl groups (smaller rim) and the secondary hydroxyl groups (larger rim) form the rims [5]. This structure possesses remarkable ability to form inclusion complexes through non-covalent interactions with guest organic and inorganic molecules of the appropriate size, shape, and polarity such as surfactant tails, drug, dyes and dendrimers [6–8].

β-CD and its derivatives are fairly rigid, flexible and their hydrophobic cavities have appropriate size [9] therefore, there are many studies on the interaction of macromolecules with β-CDs.

Surfactants are ideal guests which allow a systematic study of complexation with CD, since both their hydrophobic and hydrophilic parts can be systematically changed [10, 11]. This is why the interaction of surfactants with CDs have attracted much attention recently [2, 6].

CDs are also, able to form water-soluble inclusion complexes with many poorly water-soluble drugs, modifying their physical, chemical and biological properties [3, 12, 13]. The inclusion of a guest drug can improve its apparent solubility, physical and chemical stabilities, dissolution and bioavailability, thus making CDs very attractive drug carriers [14].

A number of factors affect complexation. Among these factors, two factors are more important than other; first the "goodness fit" between host and guest and the second factor is hydrophobic interactions [15]. Other factors are hydrogen bonding, Vander Waals interactions and release of ring strain in the CD cavity [13, 16].

Cyclodextrin forms inclusion complexes with a guest molecule mainly with 1:1 stoichiometry [5, 10, 17, 18]. When a guest molecule is bulky or long relative to the size of CD cavity, two CD molecules are usually bound to a single guest molecule to form a 2:1 CD–guest complex [1, 5, 17]. Other stoichiometries have also been found [17].

Association of surfactant with CDs has investigated using various techniques [2]. The methods are microcalorimetry

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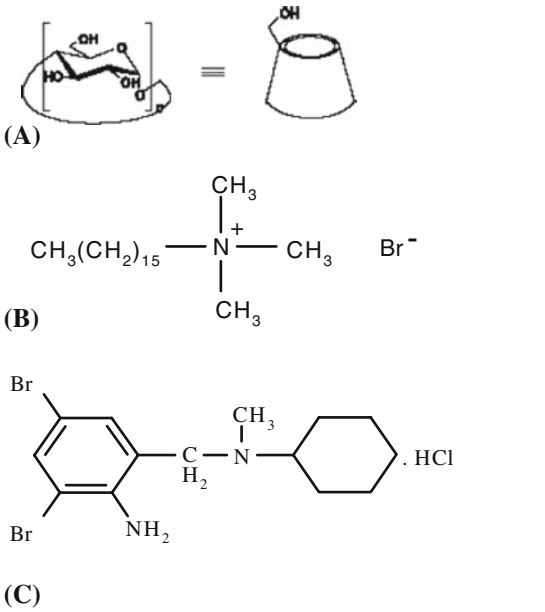

Fig. 1 Structures of cyclodextrins (**a**), HTAB (**b**) and bromhexine (**c**)

[5, 12], vapor pressure [5], surface tension [19, 20], NMR [19, 21], sound velocity [10], conductivity [5, 10, 20], UV–Vis spectroscopy [10, 21] and surfactant-selective electrode [10, 22, 23].

All the methods have their own limitation, and curve fitting procedures should always be applied with caution. Among various methods, *electromotive force* (EMF) measurements using a surfactant-selective electrode give monomer concentration of surfactant, which allows us to determine association constants directly [20].

In this work, the study of the inclusion of cationic surfactant hexadecyltrimethylammonium bromide (HTAB) (Fig. 1b) with α- and β-CD using conductivity method and surfactant membrane selective electrode in the presence and absence of bromhexine has been reported. Bromhexine (2-amino-3, 5-dibromo-*N*-cyclohexyl-*N*-methylbenzylamin hydrochloride) (BH) (Fig.1c) is a mucolytic agent used in the treatment of respiratory disorders associated with productive cough. It is also used as an adjuvant to improve the response to antibiotics in the treatment of respiratory infections [24]. We determined the stoichiometry and association constants of these complexes by potentiometry measurements. The stoichiometry, *the critical aggregation concentration* (CAC) and the monomer surfactant concentration of HTAB have also been calculated by conductivity measurements. The influence of drug on the interaction between HTAB and CDs also, investigated.

Materials and methods

Materials

Hexadecyltrimethylammonium bromide (MW = 364.46) was obtained from Merck. α-CD (MW = 972.85) and β-CD (MW = 1135) were obtained from Sigma. Bromhexine hydrochloride (MW = 412.59) was obtained from Sina Daru Company. Poly (vinylchloride) (PVC) (MW = 220000) and tetrahydrofuran (THF) were purchased from Merck. Polymeric plasticizer, Elvaloy 742 was obtained from Dupont. All other materials were of analytical reagent grade. Double distilled water was used throughout the experiments. All measurements were carried out at room temperature.

Methods

Preparation of the PVC membrane electrode

A method for preparation of surfactant-selective membrane electrode reported in our previous work [25].

EMF measurements

Potentiometric measurements were carried out with a digital pH/mV meter (pH 162 from Fanavary Tajhizat Sanjesh). The surfactant membrane electrode was used to determine the surfactant monomer concentrations by measuring their EMF relative to a commercial calomel electrode, using the following cell.

$$\text{HTAB selective electrode} \left| \begin{array}{r} \text{test solution comprising constant} \\ \text{amount of CD or/and bromhexine} \\ \text{and } 1 \times 10^{-4}(\text{M}) \text{ sodium bromide} \end{array} \right| \begin{array}{r} \text{commercial calomel} \\ \text{electrode} \end{array}$$

The electrode potential of a fresh aqueous solution reached on equilibrium typically within 1 min. Response time became faster when the HTAB concentration increased.

The calibration curve for HTAB obtained from titration of 1×10^{-4} M NaBr by 0.05 M HTAB solution. The calibration curve exhibited an inflection point at C_{HTAB} about 0.001 M, signifying the critical micelle

concentration (CMC) of HTAB. Below the CMC, the plot was linear with a slope of 58–60 mV/decade that indicates Nernstian behavior.

The experiment was then repeated by measuring the relative EMF of the surfactant electrode in the presence of a constant amount of CDs or/and bromhexine.

Conductivity measurements

Conductance measurements were made using a 644 conductometer Swiss made with a Metrohm electrode, with a cell constant of 0.75 cm^{-1}. Once the solutions are prepared and introduced into the cell and the micropipette. Specific conductivity (κ) and molar conductance (Λ) of the solution was read by conductometer. Then, the different volume of the concentrated solution was added to the cell by micropipette. The net measurement started after 3 min to assure the mixing of the compounds and the new conductivity data were read.

Results and discussion

EMF studies

Titration curves of HTAB with and without α-CD are shown in Fig. 2. Similar curves were also obtained for β-CD. The concentration of surfactant monomer decreased significantly in the presence of CDs because of the strong interaction between surfactant and CDs. As shown in Fig. 2, the obtained EMF curves in the presence of CD diverge from the Nernstian behavior at first and merge with the calibration curve when free micelles have been formed.

The association constants for 1:1 and 1:2 inclusion complexes calculated by following equations:

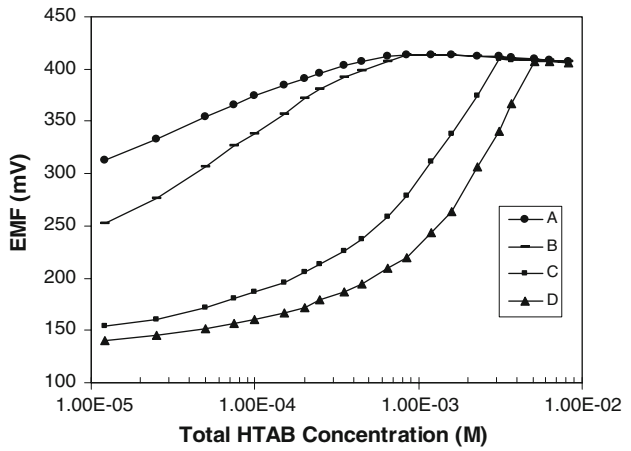

Fig. 2 EMF versus total concentration of HTAB in the presence of α-CD, [α-D] (mM): (A) 0.0, (B) 0.1, (C) 2.5, (D) 5.0

$$[CD]_{total} = [CD] + K_1[S][CD] + 2K_1K_2[S][CD]^2 \quad ((1))$$

$$[S]_{total} = [S] + K_1[S][CD] + K_1K_2[S][CD]^2 \quad ((2))$$

where [CD] is the free (uncomplexed) cyclodextrin concentration and [S] is the surfactant monomer concentration. K_1 and K_2 also are association constants for 1:1 and 1:2 complexes, respectively.

Values of K_1 and K_2 are calculated by means of a least-squares computer fitting program. The program is based on the iterative adjustment of K_1 and K_2 while difference between [S] is calculated from Eqs. 1 and 2 and the measured values using the surfactant-selective electrode are criterion for "goodness fit". This procedure is repeated in the case of each cyclodextrin concentrations. All the calculated association constants are summarized in Table 1 and compared with values in the literature [10, 22, 26, 27]. These data show that in the case of HTAB/α-CD complexes, the value of K_1 is very greater than K_2, which indicates that the formation of the 1:1 stoichiometric complex predominates rather than 1:2 ones.

The curves of interaction between HTAB and different concentration of bromhexine are shown in Fig. 3. As expected, with the increase of drug concentration, interaction increased.

The inflection has attributed with the onset of binding and usually called CAC. The difference between binding and calibration curves before CAC is due to the adsorption of drug on the membrane that affects the relative magnitude the millivolts. As shown in Figs. 2 and 3 the mechanism of the process leading to the formation of

Table 1 Association constants K_1 and K_2 (1/M) for HTAB interactions with α- and β-cyclodextrin

$[CD]_{total}$/mM	K_1	K_2
This work		
α-CD		
0.1	94520	5730
2.5	95000	1930
5.0	95240	1530
β-CD		
0.09	61080	
2.2	62010	50
4.4	62190	
Mwakibete [10]		
α-CD	99200	20400
β-CD	67700	9600
Dharmawardana [22]		
β-CD	65500	398
Jezequel [26]		
β-CD	70790	126
Park [27]		
β-CD	59800	390

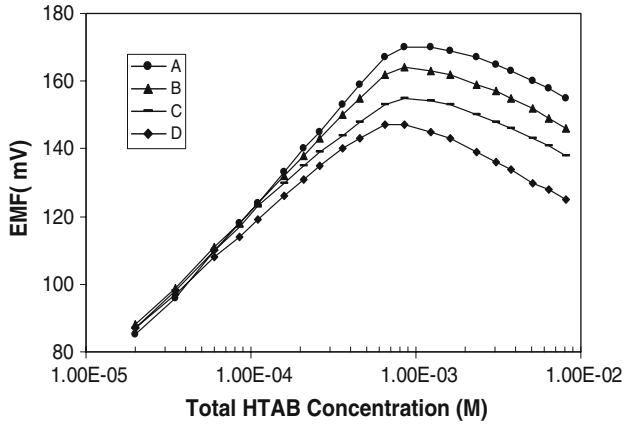

Fig. 3 EMF versus total concentration of HTAB in the presence of bromhexine, [BH] (mM) (A) 0, (B) 0.6, (C) 1.0, (D) 1.2

drug/surfactant complexes is different from the type of interaction between CDs and HTAB. Figure 3 shows that the EMF curves obtained in the presence of drug merge with calibration curve when the HTAB concentration increases until 6×10^{-5} M, is no interaction between HTAB monomers and the drug. From the HTAB concentration of 6×10^{-5} M until CMC aggregates form (dimers, trimers, …), therefore interaction occurs between the aggregates and the drug. On the other hand, there is no interaction between drug and monomers of surfactant, but after formation of surfactant aggregations, interaction starts. In the presence of CDs as can be seen in Fig. 2, because CDs have hydrophobic cavity with the appropriate size, monomers of surfactant are incorporated into CD cavity and form the inclusion complexes.

Figure 4a and b has obtained when both drug and CDs were present. Comparison between Fig. 4a and b clearly shows that the interaction between surfactant and CDs is decreased in the presence of drug. This fact suggests that both the surfactant and the drug are incorporated into CDs cavities. It is found that in the same concentration of both CDs, but different concentration of drug, the effect of drug concentration on the interaction of α-CD with HTAB is greater than β-CD, which means that the interaction between drug and α-CD is stronger.

In the same concentration of α-CD by increasing the drug concentration, interaction started at lower concentration of HTAB and binding curve is merged with calibration curve in the less concentration of HTAB. The reason for this result is because some of CDs are blocked by drug. This result indicates that the tendency of drug to α-CD is more than HTAB.

As can be seen in Fig. 4a and b, the difference between curves B and C is only in BH concentration. Figure 3 shows that with the increase of the BH concentration, the interaction between drug and HTAB also increase, therefore we expect that the C curve to have divergence from

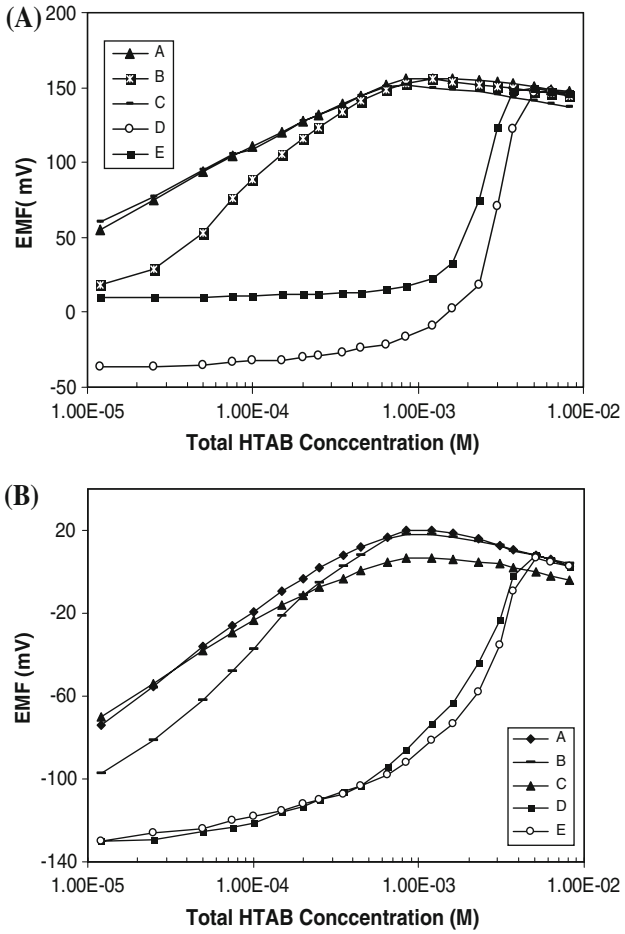

Fig. 4 (**a**) EMF versus total concentration of HTAB in α-CD/bromhexine solution (mM): (A) 0.0/0.0 (B) 0.1/0.6, (C) 0.1/1.2, (D) 5.0/0.24, (E) 5.0/1.2. 0.0/0.0 α-CD/bromhexine. (**b**) EMF versus total concentration of HTAB in β-CD/bromhexine solution (mM): (A) 0.0/0.0 (B) 0.1/0.6, (C) 0.1/1.2, (D) 4.4/0.24, (E) 4.4/1.2

calibration curve. On the other hand, as shown in Fig. 4a and b, the C curve merges with calibration curve more than B curve. It seems that in the presence of higher concentration of BH, the interaction between drug and CD increase, therefore the less drug and CD are available to interact with HTAB.

Conductivity studies

The plots of the specific conductivity (K) versus total HTAB concentration for solutions containing various concentrations of β-CD have been in Fig. 5. Similar curves were obtained for the α-CD/HTAB system. The observed inflection in all curves at a certain concentration of HTAB has generally accepted as the CMC of the formed micelles and called CAC. Inclusion complexes between surfactant and CDs are formed before the CAC. The presence of CDs always produces an increase on the CAC of the HTAB, the trend being: α-CD < β-CD.

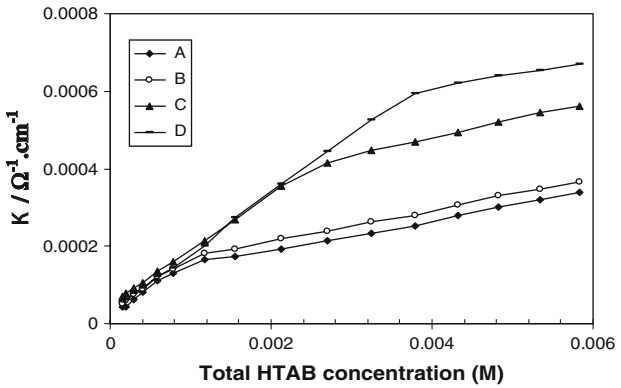

Fig. 5 Specific conductivity K for the system β-CD/HTAB versus [HTAB] at a constant [β-CD] (mM): (A) 0.0, (B) 0.1, (C) 2.2, (D) 4.4

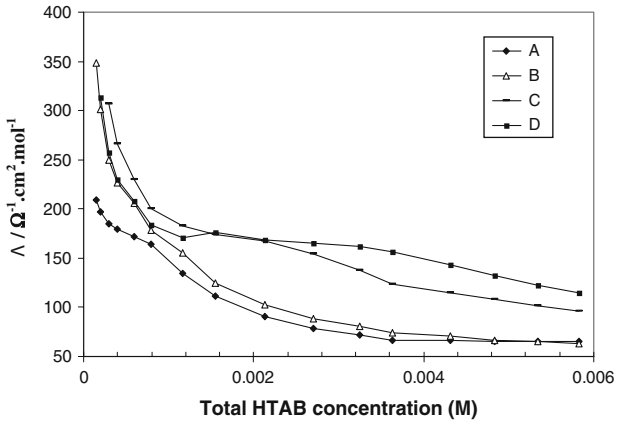

Fig. 6 Molar conductance Λ for the system β-CD/HTAB versus [HTAB] at a constant [β-CD] (mM): (A) 0.0, (B) 1.0, (C) 2.2, (D) 4.4

The association between surfactant and CD is stronger than the formation of micelles. Therefore, micelles are formed only when practically all the present CD in the solution is complexed. Figure 6 shows plots of the molar conductance, Λ, versus total HTAB concentration. Similar curves were obtained in the presence of α-CD. The observed minimum in the curves at certain [HTAB] at the presence of CD (before the micelle formation) is where inclusion complex is formed. This point is used to determine the stoichiometry of inclusion complex, $A = [CD]/[S]$, where the [CD] is the initial concentration of CD and [S] is the [HTAB] at the minimum point [4]. For complex of α-CD/HTAB, $A = 2.0 \pm 0.2$ and for β-CD/HTAB, $A = 1.0 \pm 0.2$ which means that the 1:2 and 1:1 inclusion complexes are formed between the HTAB/α-CD and HTAB/β-CD, respectively. The inner diameter cavity of the α-CD (0.5 nm) is smaller than the internal diameter of the β-CD cavity (0.6–0.7 nm). Therefore, α-CD has greater capacity to form CD-surfactant complexes with stoichiometry 1:2 [23]. The results show good agreement with results obtained from EMF measurements.

Table 2 Values of the apparent critical micellar concentration (CAC) and $[HTAB]_f$ determined from conductivity versus [HTAB] at constant [β-CD]

[β-CD] (mM)	CAC (mM)	$[S]_f$ (mM)
0.00	0.91 [28]	–
0.00	0.97 [29]	–
This work		
0.00	0.95	0.95
0.90	1.55	0.65
2.20	2.70	0.58
4.40	4.32	0.53

The results in Table 2 clearly show that inclusion complex on the micelle formation is formed. From Figs. 5 and 6 it can be determined the values of CMC for HTAB in the absence and in the presence (CAC) of CD. In this work, the CMC of HTAB in the absence of CD is equal to 0.00095 M, which is close to the obtained value from EMF measurements. This shows good agreement with the one obtained from the literatures [28, 29]. Table 2 also shows the free surfactant concentrations, $[S]_f$. These data have been calculated from the following equation [4]:

$$[S]_f = CAC - [S]_{assoc} = CAC - [CD]/A$$

where the $[S]_{assoc}$ is the concentration of complexed surfactant.

These results indicate that by increasing the concentration of complexed surfactant, $[HTAB]_{assoc}$, CAC increases but $[HTAB]_f$ decreases slowly.

Conclusions

Based on potentiometry measurements, the interaction between HTAB and α- and β-CD in the presence and absence of bromhexine were studied. The results indicated that both HTAB and bromhexine could incorporate into CDs cavities, thus in the presence of bromhexine, the interaction between HTAB and CDs decreased. The association constants and also stoichiometry of inclusion complexes in the absence of bromhexine were determined. The large constant values suggest a significant interaction between the guest and host molecules. The difference in the association constants between HTAB and α-, β-CD clearly reflects the difference in the internal diameter of the CD cavities and "goodness fit" between HTAB and CDs. By conductivity measurements, the stoichiometry of the inclusion complexes and the effect of the inclusion complexes on the micellization process of the HTAB were determined. The results showed, that the CAC increased with CD concentration, while $[HTAB]_f$ decreased slowly.

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