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Binary and Ternary Complexes Between Lauryl Hexaoxyethylene, Benzoate and Cyclodextrin. Part I. α-CD

EVA SCHNEIDERMAN and APRYLL M. STALCUP*
Department of Chemistry, University of Cincinnati, Cincinnati OH 45221-0172, USA

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Abstract

The characterization of binary and ternary complexes of benzoate, lauryl hexaoxyethylene ($C_{12}E_6$) and α-CD is presented. Complexation was characterized by capillary zone electrophoresis and titration microcalorimetry. These techniques suggested that α-CD formed two qualitatively different complexes with $C_{12}E_6$. The stoichiometric ratio of α-CD : $C_{12}E_6$ in the individual complexes was 1 : 1 and 1.4 : 1, with stability constants of 2.0×10^3 M^{-1} and 6.4×10^5 $M^{-1.4}$, respectively. At higher concentrations of interacting species (10 mM), a precipitate formed. Addition of benzoate seemed to prevent precipitate formation and appeared to increase the stability constants of both binary complexes. Both techniques also indicated formation of ternary complexes between α-CD, $C_{12}E_6$ and benzoate.

Abbreviations: α-CD – alpha cyclodextrin; $C_{12}E_6$ – lauryl hexaoxyethylene; BTP – bis-tris propane; CE – capillary electrophoresis; cmc – critical micelle concentration

Introduction

Cyclodextrin inclusion complexes are the most often described by stoichiometry and stability constants (K) [1, 2]. Various techniques have been employed to determine these characteristics including UV-Vis [3], fluorescence and phosphorescence spectroscopy [4, 5], nuclear magnetic resonance spectroscopy [6], calorimetry [7, 8], capillary zone electrophoresis [9], isotachophoresis [10], and liquid chromatography [11]. However, the results from various techniques sometimes lead to contradictory conclusions. It has been suggested that techniques providing the most consistent determination of stability constants and stoichiometry are calorimetric methods and methods providing a direct measure of the free and complexed forms of the guest and/or cyclodextrin [12].

Commonly studied cyclodextrin – nonionic guest interactions are systems involving Triton surfactants [13, 14], mixtures of thereof [15, 16] and polyrotaxanes [17–19]. Triton surfactants consist of aliphatic, oligooxyethylene, and phenyl residues. Several binding studies of Triton X-100 with α-CD were reported. These complexes were investigated via calorimetry [13], NMR spectroscopy [13, 20], X-ray crystallography [14], circular dichroism [20], UV-Vis spectroscopy [14], and surface tensiometry [14, 20]. The reported stability constants were on the order of 10^5 and the stoichiometry of these complexes was 2 : 1 (cyclodextrin : surfactant).

Less attention has been paid to studies of structurally simpler analogs of Triton surfactants such as alkyl oligooxyethylenes. Surface tension investigations and X-ray crystallographic studies were reported for the linear alkyl ethoxylate Brij 35 [14]. This surfactant has a long hydrophilic part (23 ethylene glycol residues) and its behavior, with regard to interactions with cyclodextrins, is similar to that of polyethylene glycol [14].

Titration microcalorimetry is a technique readily selected for studying molecular interactions. It is the only direct method for the determination of reaction enthalpy; however, it is not as widely used for the characterization of ligand-cyclodextrin complexation [21] as spectroscopic methods, perhaps because of solubility issues or the magnitude of the binding constants. It is used not only to determine stability constants and stoichiometry of the complex, but also to obtain information about the differences in thermodynamic quantities associated with the change in the structural features of the ligand for the members of homologous series or other structurally related compounds [22, 23]. Of course, one of the main advantages of titration microcalorimetry in the present study is that it can be used for the study of UV inactive species (e.g., $C_{12}E_6$ and α-CD).

Capillary electrophoresis (CE) is a separation technique in which compounds are separated based on the differences in electrophoretic mobilities. CE has been used in various applications for separation of small ionic compounds [24], biomolecules [25], and chiral compounds [26]. Many review articles and monographs have been published on this subject

* Author for correspondence.

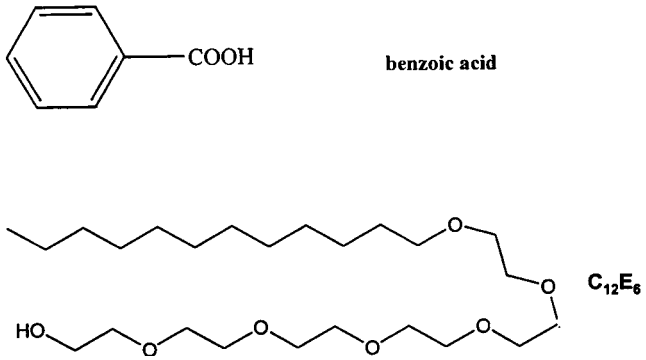

Figure 1. Structure of benzoic acid and lauryl hexaoxyethylene ($C_{12}E_6$).

[27]. The main advantage of capillary electrophoresis over titration microcalorimetry is that it is able to separate individual free and complexed species in the solution assuming relatively large stability constants and sufficient resolution power.

The structure of $C_{12}E_6$ and benzoic acid is shown in Figure 1. In preliminary experiments, it was found that alkyl oligoxyethylene $C_{12}E_6$ forms unusually strong complexes with α-CD and that benzoate appeared to increase the stability constants suggesting ternary complex formation. However, because the α-CD cavity is rather small, the formation of a ternary complex was somewhat surprising; most stable ternary complexes of α-CD contain water and another low molecular weight compound [28]. No ternary complexes were reported for α-CD and polyethylene glycol [29], fatty alcohol, Triton X-100, or Triton X-45 [13, 14, 20]. In this report, a basic description of the binary and ternary complexes between $C_{12}E_6$, α-CD, and benzoate, will be given. Two techniques, titration microcalorimetry and capillary electrophoresis, were employed in the studies to investigate complexation equilibrium.

Experimental

Materials

All chemicals were of analytical grade and used as is. For all experiments, distilled and deionized water with resistance higher than 17 MΩ was used. Benzoic acid, 6 M hydrochloric acid (AA grade), and sodium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA, USA), α-CD was purchased from Acros Organics (Pittsburgh, PA, USA), bis-tris propane (1,3-bis[trishydroxymethyl]aminopropane – BTP) and lauryl hexaoxyethylene ($C_{12}E_6$) were purchased from Sigma Chemical (St. Louis, MO, USA). The water content for α-CD was measured via Karl-Fisher titration and was accounted for in the calculation of exact concentration.

Instrumentation

Titration microcalorimetry

For calorimetric measurements, a MicroCal Omega-ITC microcalorimeter was used following standard instrumental procedures. The titration was performed with a 250 μl injection syringe and 400 rpm stirring. A multistep titration in 20–30 steps, 3–5 μl each, was performed. Steps were spaced at 150–180 s intervals. The duration of each injection was 20 s. Data was collected at 25 °C ± 1 °C.

The BTP buffer, adjusted to pH 7 with HCl, was used for microcalorimetric experiments. The concentrations of α-CD, $C_{12}E_6$, and benzoate were in the millimolar range. To determine if BTP interacted with α-CD and $C_{12}E_6$, thereby perturbing the results, calorimetry experiments were performed in 0.05 M and 0.1 M of BTP. The stoichiometry and stability constants of α-CD with $C_{12}E_6$ remained the same in both buffer concentrations, thus indicating that BTP did not interfere with analysis. For consistency, data collected in 0.1 M buffer is reported.

Complexation constants, stoichiometries, and heat enthalpies, were calculated from initial heat pulses by nonlinear regression methods using Microcal ORIGIN software. The heat of dilution of cyclodextrin and benzoate in the buffer was subtracted from the calorimetric data. The reaction enthalpy of demicellization of $C_{12}E_6$ due to the dilution was below the detection limit of the instrument. Correction for the heat of demicellization of $C_{12}E_6$ arising from the addition of cyclodextrin was not possible [30].

Capillary electrophoresis

A Bio-Rad 3000 capillary electrophoretic system equipped with a fast-scanning UV-Vis detector (Bio-Rad Laboratories, Inc., Hercules, CA, USA) interfaced to a Gateway 2000 PC was used for all CE measurements. Fused silica capillaries (75 μm ID, 350 μm OD) were also obtained from Bio-Rad Laboratories.

The carousels and the capillary were thermostatted at 25 °C. The length of the capillary was 35 cm to the detector with a total length of 39.5 cm. The separation voltage was 17 kV for all experiments. Eluting zones were detected indirectly at 224 nm. The detector was at the cathodic end of the column. Between measurements, the capillary was flushed with a mixture of 1 M NaOH in a 50% aqueous solution of CH_3OH for three minutes, water for three minutes, and a run buffer for four minutes.

To determine free α-CD in the binary mixtures of α-CD and $C_{12}E_6$, the binary mixture was injected as a sample. To assure equilibration, samples were prepared at least 6 hours before an experiment. Six separation buffers that differed in the concentration of benzoate (10, 20, 30, 40, 50, 60 mM) were used. The buffer pH was adjusted to 7.0 with BTP. Calibration standards were 1–6 mM solutions of α-CD in water. Corrected peak areas (peak area/retention time) were plotted against concentration of α-CD in a standard sample. All calibration curves were linear in the investigated concentration range in all buffers used. Hydrostatic injection

(10 psi.sec) was used for analysis in buffers containing 10, 20, and 30 mM benzoate; a 15 psi.sec injection was used for buffers containing 40 and 50 mM benzoate and a 20 psi.sec injection for the buffer containing 60 mM benzoate. Longer injection times in the buffers with higher concentration of benzoate were necessary to assure precision in the peak area integration. Two sets of samples were prepared. In the first set of experiments, the concentration of $C_{12}E_6$ in the sample was kept constant, while the concentration of cyclodextrin was varied. In the second set of experiments, the concentration of cyclodextrin in the samples was kept constant, while the concentration of $C_{12}E_6$ was varied. Both sets of experiments gave consistent results.

Results and discussion

Titration microcalorimetry

In the experimental set-up, a solution of α-CD or a mixture of α-CD and benzoate, respectively, was step-wise injected into a solution of $C_{12}E_6$ under constant pressure. The heat released, dQ, after each injection was measured and converted into a dependence of released heat as a function of molar ratio of α-CD/$C_{12}E_6$ (binding isotherm). The reaction enthalpy, complex stoichiometry, and equilibrium constants may be determined from the binding isotherm. For the sake of brevity only the expressions for a 1 : 1 complex will be shown [31].

It can be shown that mass balance and stability constant equations yield the following expression relating stability constant, K, and initial and equilibrium concentrations of cyclodextrin c_{CD}, [CD], surfactant c_S, [S], and the cyclodextrin-surfactant complex, [CD-S], respectively.

$$[\text{CD-S}]^2 + [\text{CD-S}]^* \left(-c_{CD} - c_S - \frac{1}{K} \right) + c_{CD} c_s = 0. \quad (1)$$

Upon addition of cyclodextrin to a solution containing surfactant, the change in the equilibrium concentration of the complex, [CD-S], can be expressed in term of the heat released, dQ,

$$dQ = d[\text{CD-S}]^* \Delta H^* V_0 \quad (2)$$

in which ΔH is the reaction enthalpy and V_0 is the initial volume.

Substitution of (1) into (2) and rearrangement yields (3).

$$\frac{1}{V_0} * \frac{dQ}{dc_{CD}} = \Delta H$$

$$* \left(\frac{1}{2} + \frac{1 - (1+r)/2 - \frac{c_{CD}}{2 * c_S}}{\sqrt{\frac{c_{CD}^2}{c_S^2} - 2 * \frac{c_{CD}}{c_S} * (1-r) + (1+r)^2}} \right), \quad (3)$$

where

$$r = \frac{1}{K c_S}. \quad (4)$$

According to this relationship, the heat exchanged during one injection step is a function of the initial concentrations of cyclodextrin and the surfactant as well as the stability constant.

An iterative procedure is used to find a solution to equation (3) by assuming initial values for K and ΔH and comparing calculated values of ΔQ with experimentally determined values, until no further improvement in the fit of the experimental data. Because ΔH and K can be determined directly in a single experiment, ΔG (apparent free energy) and ΔS (entropy) may then be calculated:

$$\Delta G = -RT \ln K = \Delta H - T \Delta S. \quad (5)$$

The binding isotherm for the α-CD-$C_{12}E_6$ system can be seen in Figure 2. The top part of this figure represents individual injections of α-CD into a solution containing $C_{12}E_6$. The bottom part of this figure shows the experimental (squares) and theoretically fitted (full line) binding isotherm. The fit of the experimental data indicated that two complexes were formed. In the first complex (**1**), the ratio of α-CD and $C_{12}E_6$ was 1 : 1 with a stability constant 2.0×10^3 $M^{-1} \pm 0.2 \times 10^3$. This value is consistent with values obtained for fatty acids and fatty alcohols [34], suggesting inclusion of the alkyl chain.

In the second complex (**2**), the stochiometric ratio of α-CD : $C_{12}E_6$ was 1.4 : 1.0 and the stability constant 6.4×10^5 $M^{-1.4} \pm 0.8 \times 10^5$. The higher value of the stability constant for **2** is consistent with stability constants of higher order complexes of molecules containing oligooxyethylene residues [13, 14, 20] and α-CD. In a calorimetric study [13] of α-CD with Triton X-100, the apparent stability constant for a higher order complex (1 : 2) was found to be on the order of 10^5. Topchieva *et al.* [14] also reported that the stoichiometry of the interaction between Triton X-45 and α-CD was 1 : 2. The higher stoichiometry of **2**, analogous to the reported complex of α-CD : Triton X-100, and α-CD : polyoxyethylene, suggested threading of α-CD onto the surfactant chain and could explain this larger stability constant [17–19].

An apparent free energy ΔG for the complex can be calculated from $\ln K$. The apparent ΔS can be calculated from the experimentally determined ΔH and calculated ΔG. Figure 3 provides a comparison of the relative contribution of ΔH and ΔS to the binding of **1** and **2**. It is important to note that these quantities are only apparent because they are based on concentrations rather than activities. As can be seen in Figure 3, both complexes appear to be enthalpy-driven, perhaps due to the release of water molecules from the cavity [32]. However, whether formation of hydrogen bonds or desolvation of lipophilic surfaces is the major contributor to a formation of these complexes is still a subject open to debate [14, 33]. In both complexes, entropy negatively contributes to the stability of the complex. Decreased flexibility upon binding and the unique structure of water, can both contribute to the entropy changes [14, 34, 35]. The negative

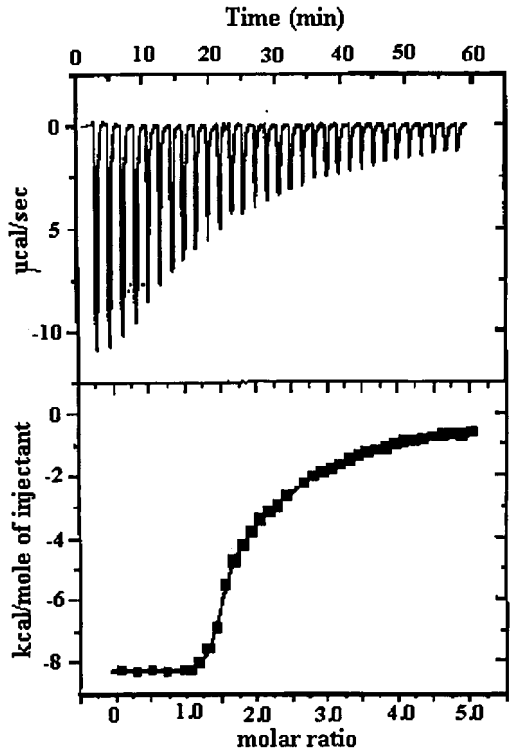

Figure 2. Binding isotherm of α-CD and $C_{12}E_6$. $C_{12}E_6$ (0.48 mM) was titrated with α-CD (15.5 mM) in 0.1 M BTP buffer (pH = 7.0).

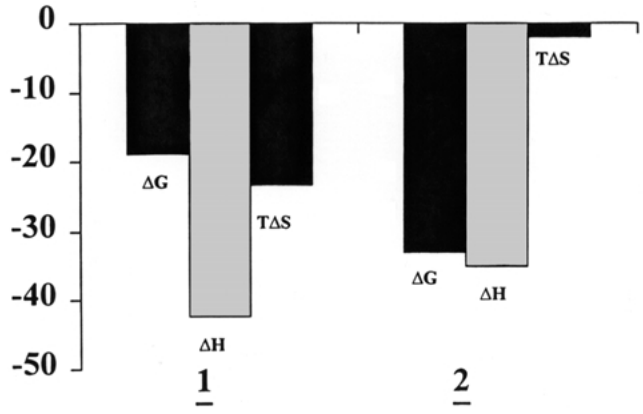

Figure 3. Free energy (ΔG), enthalpy (ΔH), and entropy ($T\Delta S$) for two complexes of α-CD with $C_{12}E_6$ in buffered aqueous solutions (pH = 7.0) at 25 °C.

contribution of entropy in **1** appears to be more significant than that of complex **2**, which suggests that the flexibility of $C_{12}E_6$ in **1** may be different than the flexibility in **2**. The network of H-bonds of water molecules surrounding these complexes can also be qualitatively different.

At α-CD and $C_{12}E_6$ concentrations higher than 5 mM, a precipitate formed. Addition of benzoate appeared to enhance the solubility of the precipitate. Figure 4a shows the dependence of the stability constant K_1 of **1** (α-CD : $C_{12}EO_6$ = 1 : 1) on the ratio of benzoate : α-CD in the ligand solution. As can be seen from the Figure 4a, the stability constant increased until the molar ratio of benzoate : α-CD was approximately equal to one. No significant increase in the stability constant K_1 was observed beyond this point. The stability constant K_2 (Figure 4b) appeared to increase until

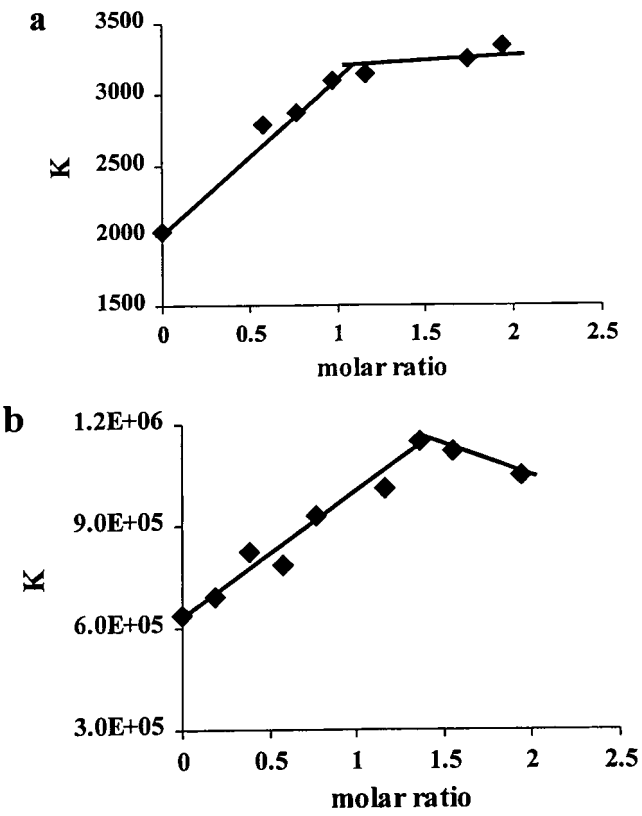

Figure 4. A plot of (a) the stability constant K_1 of complex **1** (α-CD : $C_{12}E_6$ = 1 : 1) and (b) the stability constant K_2 of complex **2** (α-CD : $C_{12}EO_6$ = 1.4 : 1) vs. molar ratio of benzoate : α-CD.

the molar ratio of benzoate : α-CD was about 1.5. The stability constant increase of α-CD and $C_{12}E_6$ as a function of the benzoate concentration, followed by leveling off suggested stoichiometric interaction of both binary complexes with benzoate.

Capillary zone electrophoresis

As was shown by the titration microcalorimetry experiments, $C_{12}E_6$ strongly complexes with α-CD. Because of the high stability constant of **2**, CE experiments were performed in hopes of separating free from complexed α-CD in binary mixtures of α-CD and $C_{12}E_6$. A modified method for the analysis of α-, β-, and γ-cyclodextrins was implemented [36]. A crucial component of the separation buffer was benzoate. Benzoate served as both a chromophore for indirect detection of the UV-inactive cyclodextrin, and also provided a discriminating media for free α-CD and α-CD complexed with $C_{12}E_6$.

Figure 5a is the electropherogram obtained from an injection of α-CD in water using inverse detection. The first peak (labeled as A) corresponds to water; the second peak (labeled as C) corresponds to free α-CD. Complexation with benzoate confers a partial negative charge on the complex, which emerges after the neutral zone of water. Figure 5b is the electropherogram obtained from the injection of $C_{12}E_6$ in water. Under the experimental conditions, $C_{12}E_6$ was neutral; thus, if no strong interaction occurred with ben-

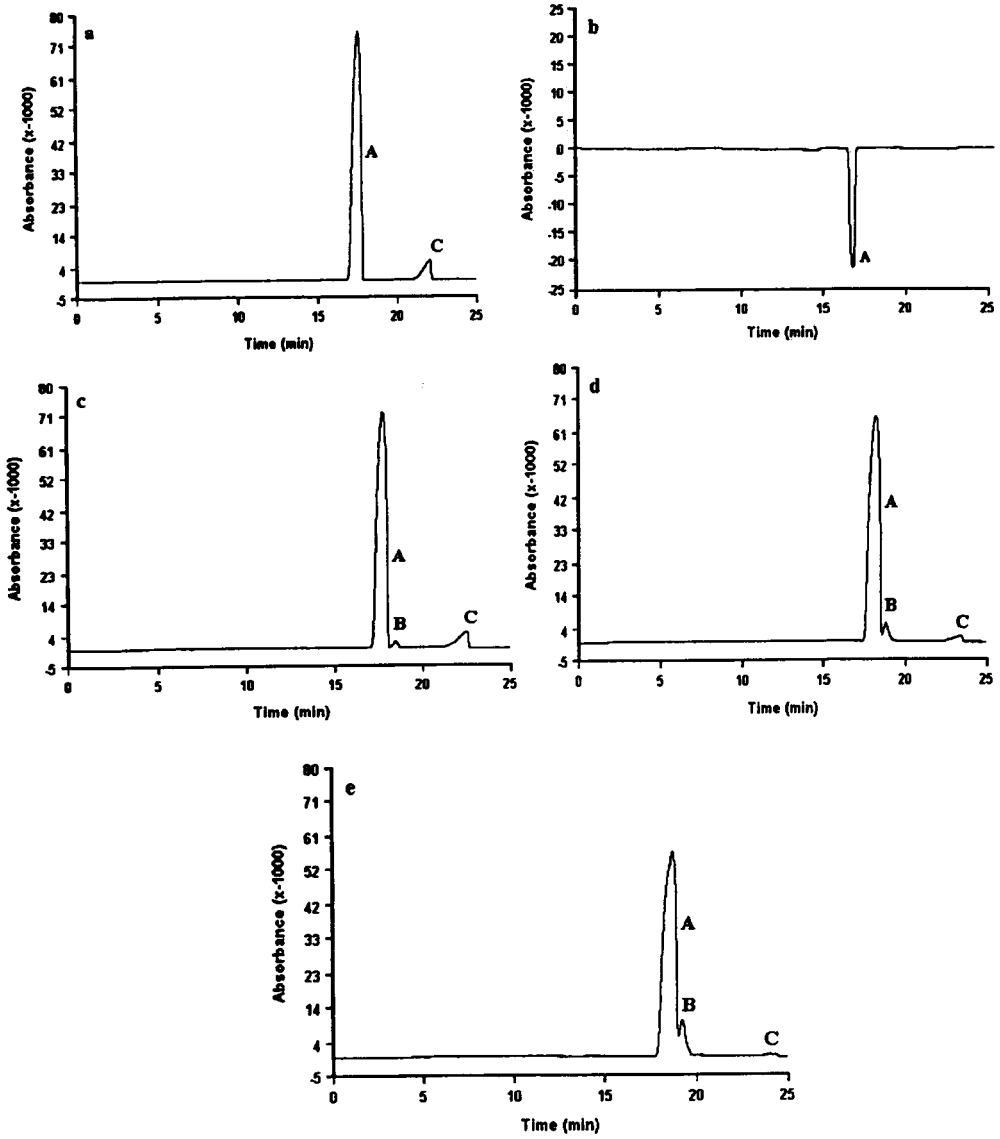

Figure 5. Electropherograms of analysis of α-CD, $C_{12}E_6$, and mixtures of α-CD with $C_{12}E_6$; (a) α-CD; (b) $C_{12}E_6$; (c) molar ratio of α-CD: $C_{12}E_6$ = 1 : 1, (d) molar ratio of α-CD : $C_{12}E_6$ = 2 : 1, (e) molar ratio of α-CD : $C_{12}E_6$ = 1 : 10. Separation buffer 20 mM benzoic acid adjusted to pH = 7 with BTP. U = 17 kV, L = 35 cm to a detector.

zoate, $C_{12}E_6$ should elute in the neutral zone. As Figure 4b shows, elution of only one peak was observed. Figure 5c shows the corresponding electropherogram of an aqueous mixture containing CD and $C_{12}E_6$ in a molar ratio equal to 10 : 1. As can be seen in Figure 5c, the presence of $C_{12}E_6$ induces the appearance of a new peak (B). Figure 5d shows the electropherogram for a mixture of α-CD : $C_{12}E_6$ in a molar ratio of 2 : 1. As can be seen in Figure 5d, as the concentration of $C_{12}E_6$ in the sample increased, the area of peak B also increased, while the area of peak C (α-CD) decreased. Figure 5e shows the electropherogram of a mixture of α-CD:$C_{12}E_6$ equal to 1 : 10. In this figure, the peak corresponding to free α-CD (peak C) has almost disappeared. It is important to note that partial resolution of the binary complex between α-CD with $C_{12}E_6$ from the zone of neutral components would not be possible without interaction of the binary complex with benzoate. The fact that the peak area of the binary complex (peak B) did not change when a large excess of $C_{12}E_6$ was added to the sample (data not shown) also suggests stochiometric binding of benzoate with the binary complex.

Conclusions

Titration microcalorimetry established that α-CD forms two binary complexes with $C_{12}E_6$. Dependence of the stability constants of both complexes (**1** and **2**) on benzoate concentration in the buffer, and separation of binary complexes **1** and **2** in CE experiments evidenced formation of ternary complexes between α-CD, $C_{12}E_6$, and benzoate. However, the structure of the complexes could not be derived from the techniques investigated and will be the subject of future investigations.

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