Carbohydrate Polymers 110 (2014) 472–479

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Cyclodextrin–benzoic acid binding in salt solutions: Effects of biologically relevant anions

I.V. Terekhova*, E.S. Chibunova, R.S. Kumeev, G.A. Alper

G.A. Krestov Institute of Solution Chemistry of Russian Academy of Sciences, 1 Akademicheskaya Street, 153045 Ivanovo, Russian Federation

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form 11 April 2014
Accepted 16 April 2014
Available online 26 April 2014

Keywords:
Cyclodextrin
Complex formation
Salt effects

ABSTRACT

Inclusion complex formation of benzoic acid with α-, β- and γ-cyclodextrins in water and in 0.2 M solutions of inorganic salts (KCl, KBr, KH_2PO_4 and K_2SO_4) has been studied by means of ^1H NMR at 298.15 K. Binding constants have been determined and role of biologically active inorganic anions in the inclusion complex formation has been revealed. It has been shown that effects of the anions are determined not only by changing the ionic strength. More pronounced influence of Br^- and $H_2PO_4^-$ compared with Cl^- and SO_4^{2-} is caused by specific ion-molecular interactions, occurrence of which depends on the physical–chemical properties of the anions as well as on the binding mode of cyclodextrins with benzoic acid. Competing interactions of cyclodextrin-anion were observed in the presence of KBr, while the ternary complex formation was detected upon addition of KH_2PO_4.

1. Introduction

Application of cyclodextrins as drug carriers and encapsulating agents in the pharmaceutical field is well established (Brewster & Loftsson, 2007; Del Valle, 2004; Uekama, 2002) and based on the following facts. Firstly, cyclodextrins (CDs) are cyclic oligosaccharides obtained by the enzymatic degradation of starch and composed of glucose units. Consequently, they are nontoxic for the living organisms at low oral dosages. Secondly, CDs possess a cone-shaped inner cavity, which is able to include drugs and biologically active molecules. Inclusion complexes are formed *via* noncovalent interactions and display only moderate stability ($K < 10^5$). Therefore, the encapsulated guest can be delivered to the target, and then it can be easily released and penetrate through the cell membrane. The main advantages of the encapsulation by CDs are the improvement of drug properties (*e.g.*, solubility, taste, stability) (Brewster & Loftsson, 2007), prolongation of its therapeutic action (Uekama, 2002), and the elimination of unwanted side effects (Del Valle, 2004). It is evident that investigation of CD complex formation and behavior of the inclusion complexes in solutions imitating the internal environment of the body is of practical importance.

Our research was designed to study on the role of biologically relevant inorganic salts in the inclusion complex formation of CDs with some aromatic carboxylic acids. Recently, we examined the effects of monovalent cations K^+ and Na^+ (Romanova, Chibunova, Kumeev, Fedorov, & Terekhova, 2013; Terekhova, Romanova, Kumeev, & Fedorov, 2010) as well as the anions Cl^- and Br^- (Terekhova, Chibunova, Kumeev, & Alper, 2013) in complex formation of α-CD with nicotinic, aminobenzoic and benzoic acids. It has been shown that effects of K^+ and Cl^- are negligible while the influence of Na^+ and Br^- is more pronounced and it is exhibited in the decrease of stability constants of the inclusion complexes. It has been demonstrated that the ionic pairing between Na^+ and ionized carboxylic acid group of the acids as well as the penetration of Br^- anions into α-CD cavity are the main processes which affect the equilibrium of α-CD/carboxylic acid inclusion complex formation.

These aspects motivated our present work, where the role of different biologically important anions (Cl^-, $H_2PO_4^-$, Br^- and SO_4^{2-}) in complex formation of α-CD, β-CD and γ-CD with benzoic acid (BA) are studied. Herein, salt effects in the binding of β-CD and γ-CD with BA will be reported for the first time. Dependence of the salt effects on the structure and properties of the anions as well as the size of CD cavity will be analyzed. It should be mentioned that native CDs were chosen to exclude the additional influence of substituents on complex formation process. Choice of BA as a guest molecule is determined by (a) its ability to participate in the inclusion complex formation with all cyclodextrins under consideration; (b) widespread application as medicine or constituent of marketed and developmental drugs as well as preserving agent E210 (Shtenberg & Ignat'ev, 1970; Zhang et al., 2010). Therefore, the obtained results can be interesting and useful not only for the fundamental science but also for the pharmaceutical, food and

* Corresponding author. Tel.: +7 910 984 10 94; fax: +7 4932 336237.
E-mail address: ivt@isc-ras.ru (I.V. Terekhova).

http://dx.doi.org/10.1016/j.carbpol.2014.04.057
0144-8617/

cosmetic industries where the complexed forms of biomolecules and drugs are successfully used.

2. Materials and methods

2.1. Materials

Benzoic acid, α-CD, β-CD, γ-CD, KCl, KH_2PO_4 and K_2SO_4 were Sigma-Aldrich reagents, while KBr was from Acros Organics. All of them were of analytical grade and used without further purification. The solutions were freshly prepared using doubly distilled water. The digital pH-meter (Mettler Toledo FiveEasy) was used to monitor the pH of the solutions.

2.2. ^1H NMR

^1H NMR spectra were recorded on a Bruker-AV-500 spectrometer operating at 500 MHz and temperature of 298.15 K. Chemical shifts are expressed in ppm. Cyclohexane was used as external reference to avoid the complex formation of standard with CDs. Deuterated water (99.9%) was used as solvent.

To study complex formation of CDs with BA, the ^1H NMR spectra of CDs were registered at constant CD concentration (0.005 mol/kg) and variable BA concentration (0–0.02 mol/kg) in water and in 0.2 M solutions of the salts. To investigate CD-anion binding, the ^1H NMR spectra of CDs were recorded at constant CD concentration (0.005 mol/kg) and changeable concentration of the salts (0–1.5 mol/kg). Concentration dependences of the chemical shift changes of CD protons were used to obtain binding constants (K) by the nonlinear least-squares method.

2.3. Solubility measurements

Saturated BA solutions were prepared by adding an excess amount of BA to pure water or to salt solutions with concentrations up to 1.0 mol/kg. Solutions were stirred at room temperature for 2 days. Preliminary experiments showed that this time is sufficient to achieve equilibrium. Solutions were then filtrated and analyzed spectrophometrically using UNICO 2800 spectrophotometer. Each result was the average of three repeat experiments.

3. Results and discussion

Complex formation of α-CD, β-CD and γ-CD with BA in water and in 0.2 M aqueous solutions of KCl, KBr, KH_2PO_4 and K_2SO_4 was studied at 298.15 K by means of ^1H NMR spectroscopy. Binding of CDs with BA in pure water has been earlier investigated in detail (Bergeron, Channing, & McGovern, 1978; Kamiya, Mitsuhashi, Makino, & Yoshioka, 1992; Lu, Hu, Yu, & Meng, 2000; Salvatierra, Jaime, Virgili, & Sanchez-Ferrando, 1996; Siimer & Kurvits, 1989; Simova & Schneider, 2000; Terekhova, 2011). According to the results of other groups (Bergeron et al., 1978; Kamiya et al., 1992; Lu et al., 2000; Siimer & Kurvits, 1989; Simova & Schneider, 2000) and our previous results (Terekhova, 2011), α-CD and β-CD form with BA inclusion complexes of 1:1 stoichiometry. It has been observed that insertion of BA molecule into α-CD is shallow, and only a part of the aromatic ring with the carboxylic acid group are placed inside the macrocyclic cavity (Simova & Schneider, 2000). Deeper penetration of BA into β-CD cavity results in location of whole benzene ring in the macrocyclic interior (Lu et al., 2000; Salvatierra et al., 1996). Binding of γ-CD with BA is accompanied by 1:2 (host:guest) complex formation (Siimer & Kurvits, 1989). These binding modes were used for estimation of the binding constants, values of which are summarized in Table 1.

Table 1
Binding constants for complex formation between CD and BA in water and in 0.2 M salt solutions at 298.15 K[a].

Complex	H_2O	KCl	KBr	KH_2PO_4	K_2SO_4
α-CD/BA (1:1)	897 ± 20	862 ± 20	773 ± 19	599 ± 18	990 ± 15
β-CD/BA (1:1)	390 ± 25	423 ± 26	331 ± 22	636 ± 17	372 ± 28
γ-CD/BA (1:2)	6280 ± 263	7283 ± 331	5564 ± 225	6750 ± 154	7720 ± 138

[a] Apparent K with the exception of K^0 reported for complex formation in water.

It should be mentioned that for complex formation in pure water K values obtained in this work (Table 1) are in a good agreement with those available in literature for α-CD ($K = 800 \pm 83$ (Bergeron et al., 1978) and 909 ± 83 (Siimer & Kurvits, 1989)) and β-CD complexes ($K = 372 \pm 45$ (Kamiya et al., 1992) and 370 ± 27 (Siimer & Kurvits, 1989)).

Comparative analysis of the results reported in Table 1 shows that binding constants are changed in the presence of the salts. First of all, the observed variation can be caused by the changes in the ionic strength and activity coefficients. As it has been demonstrated by Gelb, Schwartz, Radeos, and Laufer (1983), the activity coefficients of pure CD and CD complex are approximately equal. Therefore, relationship between thermodynamic and apparent binding constants (K^0 and K_{app}, respectively) is mainly determined by the BA activity coefficient (γ_{BA}):

$$K^0 \cdot \gamma_{BA} = K_{app} \quad \text{for1 : 1 complex formation} \tag{1}$$

$$K^0 \cdot \gamma_{BA}^2 = K_{app} \quad \text{for1 : 2 complex formation} \tag{2}$$

The tendency of γ_{BA} to change in the presence of the salts can be estimated from the solubility experiments. In order to reveal the salting-in or salting-out effects, the BA solubility was measured in pure water and in salt solutions. Solubility diagrams are shown in Fig. 1. Solubility of BA in water at room temperature was determined as 0.0275 mol/kg. This value is consistent with $S_{BA} = 0.0279$ M reported by Yurquina, Manzur, Brito, Manzo, and Molina (2003) and with $S_{BA} = 0.028$ M obtained by Strong, Neff, and Whitesel (1989).

As may be seen from Fig. 1, BA solubility is increased under addition of KH_2PO_4, while it is decreased in the presence of KCl, KBr and K_2SO_4. Salting-in effect of KH_2PO_4 can be caused by (a) reorganization of water molecules and their participation in H-bonding with BA carboxylic acid group; (b) possible interactions of $H_2PO_4^-$ anions with —COOH group of BA. Salting-out effect of KCl, KBr and K_2SO_4 is caused by the change in the orientation of water molecules toward the BA molecule. It was demonstrated

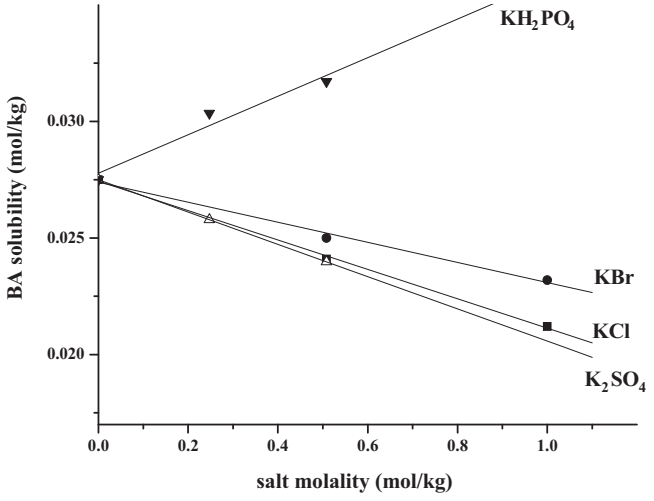

Fig. 1. Solubility of BA in the presence of the salts.

Table 2
Properties of anions in aqueous solution at 298.15 K (Marcus, 2012).

Anion	r (nm)[a]	V^{∞} (cm^3/mol)[b]	$-\Delta_{hydr}H^{\infty}$ (kJ/mol)[c]
Cl^-	0.181	23.3	367
Br^-	0.196	30.2	336
$H_2PO_4^-$	0.200	34.6	522
SO_4^{2-}	0.230	25.0	1138

[a] Ionic radius.
[b] Standard partial molar volume.
[c] Standard molar enthalpy of hydration.

that salting-out effect depends on the anion nature and decreases in the order: $SO_4^{2-} > Cl^- > Br^-$. The revealed sequence followed the Hofmeister series, according to which the anion with a higher salting-out ability has stronger interaction with water molecules and more negative energy of hydration. Thus, highly charged and more compact SO_4^{2-} is strongly hydrated as compared with Cl^- and Br^- (Table 2).

As it can be easily estimated from the solubility diagrams (Fig. 1), BA solubility is decreased by 3.6, 4.5 and 5.0% in the presence of 0.2 M KCl, KBr and K_2SO_4, respectively. On the contrary, BA solubility is enhanced 6.9% in the presence of 0.2 M KH_2PO_4. Using these values and taking into account the impact of ionic strength as a single factor affecting the inclusion complex formation one can predict the apparent binding constants on the basis of Eqs. (1) and (2). Calculated K_{app} are given in table of Support Information (SI). Fig. 2 summarizes the calculated and experimental K_{app} values for binding of BA with α-CD, β-CD and γ-CD in salt solutions. More significant differences between calculated and experimental K_{app}, which is outside the error limits, are observed for systems containing KBr and KH_2PO_4.

The obtained results (Table 1) show that binding affinity of CDs to BA is decreased in the presence of KBr despite the fact that this salt acts as salting-out reagent. Thereby, an expected increasing K_{app} was not observed, and effect of KBr was directly opposite. This can be caused by the additional contribution from possible interactions BA-ion or/and CD-ion. It has been demonstrated (Terekhova et al., 2010) that attraction of potassium cations to carboxylic acid group is very weak. In addition, cations are not included into CD cavity (Fu, Liu, & Guo, 2002; Kano, Tanaka, Minamizono, & Kawakita, 1996) as well as the interaction between BA and Br^- is unlikely. However, weak complex formation of Br^- with α-CD and β-CD takes place and it has been well documented (Matsiu, Ono, & Tokunaga, 1997; Mochida, Kagita, Matsui, & Date, 1973; Rohrbach, Rodriguez, Eyring, & Wojclk, 1977; Spencer, He, Ke, & Wu Zh Fetter, 1998; Terekhova et al., 2013; Wojcik & Rohrbach, 1975). Values of binding constants for α-CD/Br^- complexation reported by different authors lie in the range of 0.36–6.5 M^{-1} (Gelb et al., 1983; Matsiu et al., 1997; Mochida et al., 1973; Rohrbach et al., 1977; Spencer et al., 1998; Wojcik & Rohrbach, 1975). According to the binding mode proposed Matsiu et al. (1997) on the basis of ^{1}H NMR experiments, Br^- anions are located inside the macrocyclic cavity nearby the H-5 protons of CDs. It should be mentioned herein that the literature data concerning complex formation of Cl^- with α-CD and β-CD are rather contradictory. For instance, some authors discount the existence of complex formation between Cl^- and CDs (Matsiu et al., 1997; Spencer et al., 1998; Wojcik & Rohrbach, 1975). On the contrary, binding constants K varying from 1.3 to 42.1 M^{-1} were determined for complex formation of Cl^- with α-CD and β-CD by Mochida et al. (1973), Yi, Zhao, Huang, Chen, and Yu (1999), Sanemasa, Fujiki, and Deguchi (1988) and Santos, Ribeiro, Veríssimo, Lobo, and Esteso (2013). A similar situation obtains with SO_4^{2-} and $H_2PO_4^-$ anions. On the one hand, the absence of CD complex formation with SO_4^{2-} and $H_2PO_4^-$ is described by others (Matsiu et al., 1997; Yi et al., 1999). On the other hand, Taraszewska and Wójcik (1993) describe weak complexation of β-CD with these anions. Probably, the revealed discrepancy in these results can be explained in terms of different experimental methods used to study quite weak binding.

Taking into account the available literature data, we further investigated the interactions of anions with α-CD, β-CD and γ-CD by ^{1}H NMR spectroscopy. ^{1}H NMR is a proper technique for characterization of CD inclusion complexes in solutions and accurate determination of the binding constants (Schneider, Hacket, & Rüdiger, 1998). For this purpose, ^{1}H NMR spectra of CDs were recorded in salt solutions of variable concentration. The partial ^{1}H NMR spectra of CDs are depicted in Fig. 3 (for β-CD) and given in SI (for α-CD and γ-CD). Concentration dependences of chemical shift changes of CD protons are shown for some systems in Fig. 4.

It is interesting to note that addition of KBr, KCl or K_2SO_4 results in each case in downfield shift of the signals of all CD protons ($\Delta\delta > 0$), while KH_2PO_4 induces upfield shifts ($\Delta\delta < 0$) (Fig. 3). Likewise, more pronounced shifting of signals of H-5 protons upon KBr addition can be seen from Fig. 3. As follows from Fig. 4, the linear dependences of $\Delta\delta$ on salt concentration are observed for all CD protons except the H-5 protons in the presence of KBr. For H-5 protons of α-CD, β-CD and γ-CD in solutions with KBr, values of $\Delta\delta$ were slightly higher and concentration dependences in this case deviate from linearity. This result shows the inclusion of only Br^- anions into CD cavity and their location near to H-5 protons.

These results indicate that CDs selectively interact with anions under study. Complex formation with anions is realized in accordance with the principles of geometric and energetic complementarity. Table 2 lists some properties of inorganic anions in aqueous solution. Analysis of these data shows that inclusion of Br^- as a bulky and less hydrated anion is more favorable. Moreover, the binding affinity of CDs toward the Br^- anions is also determined by the cavity dimensions. The experimental results presented in Fig. 4 indicate the decrease of $\Delta\delta$ values of H-5 protons with increasing cavity diameter. Besides, the concentration

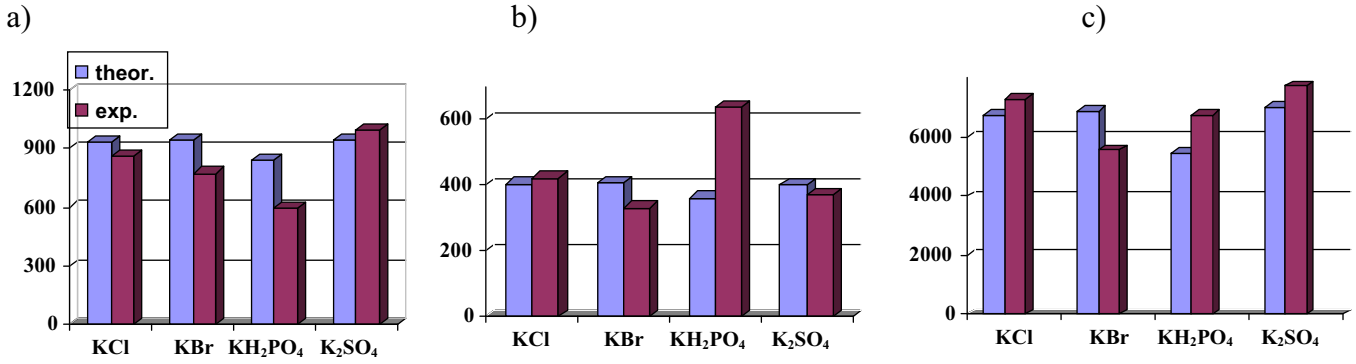

Fig. 2. Predicted and experimental binding constants for complexes of BA with α-CD (a), β-CD (b) and γ-CD (c) in salt solutions.

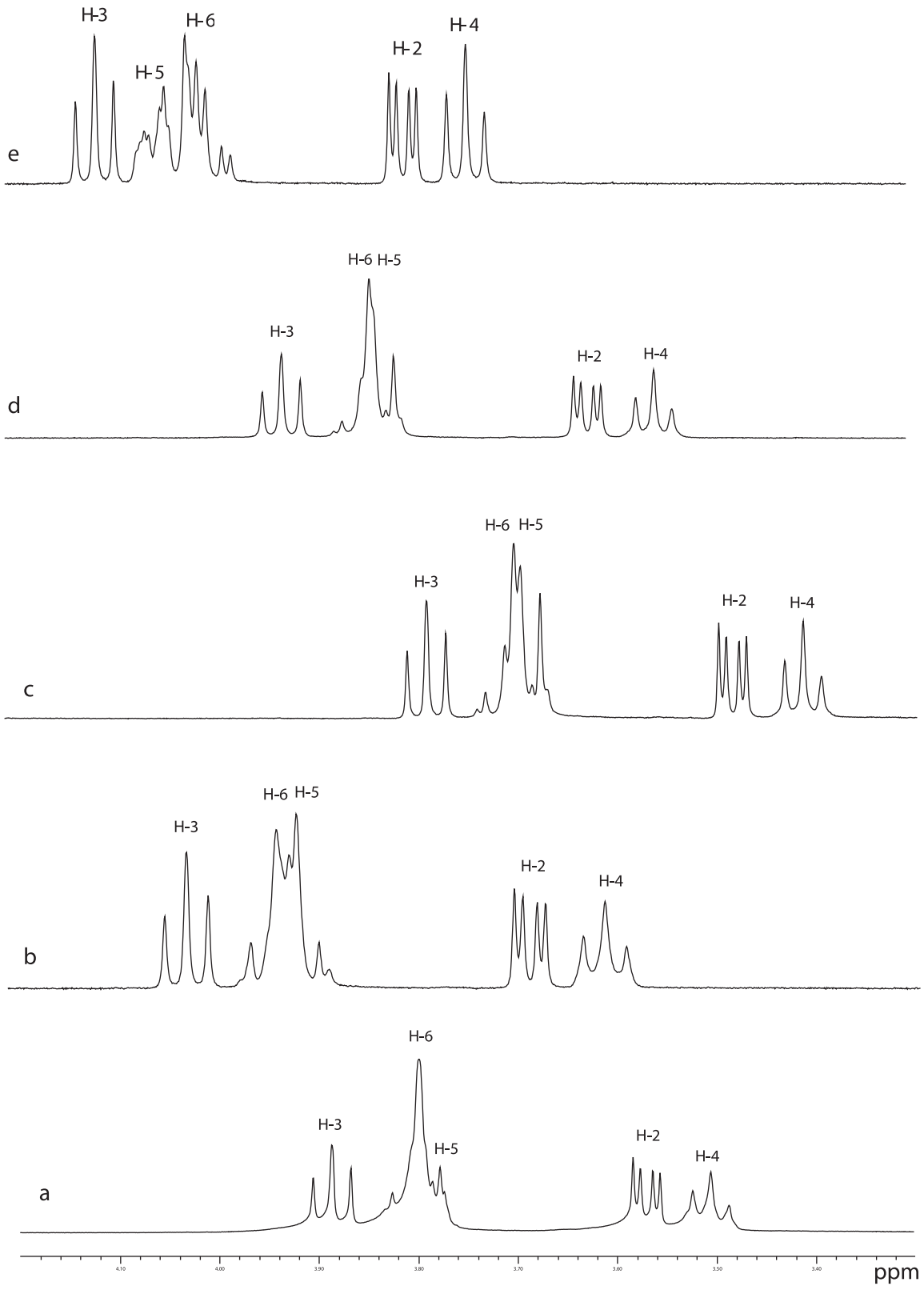

Fig. 3. Partial ^{1}H NMR spectra of β-CD alone (a) and in the presence of 1 M KCl (b), KH_2PO_4 (c), K_2SO_4 (d) and KBr (e) at 298.15 K.

dependence of $\Delta\delta$ of H-5 protons becomes nearly linear for interaction of KBr with γ-CD. We have attempted to calculate the binding constants for complexes of CDs with Br^-. These values were found to be 0.24 ± 0.05, 0.17 ± 0.03 and 0.18 ± 0.03 for α-CD, β-CD and γ-CD, respectively. Thus, higher stability of α-CD/Br^- inclusion complexes was observed. Insertion and location of Br^- inside the smallest cavity of α-CD is more tight, and van der Waals and hydrophobic interactions predominate in this case (Matsiu et al., 1997). As it is well known, van der Waals forces weaken with an increase of the cavity diameter and, as a consequence, the distance between the interacting species. We propose that this is the reason that the binding of Br^- with β-CD and γ-CD is weaker.

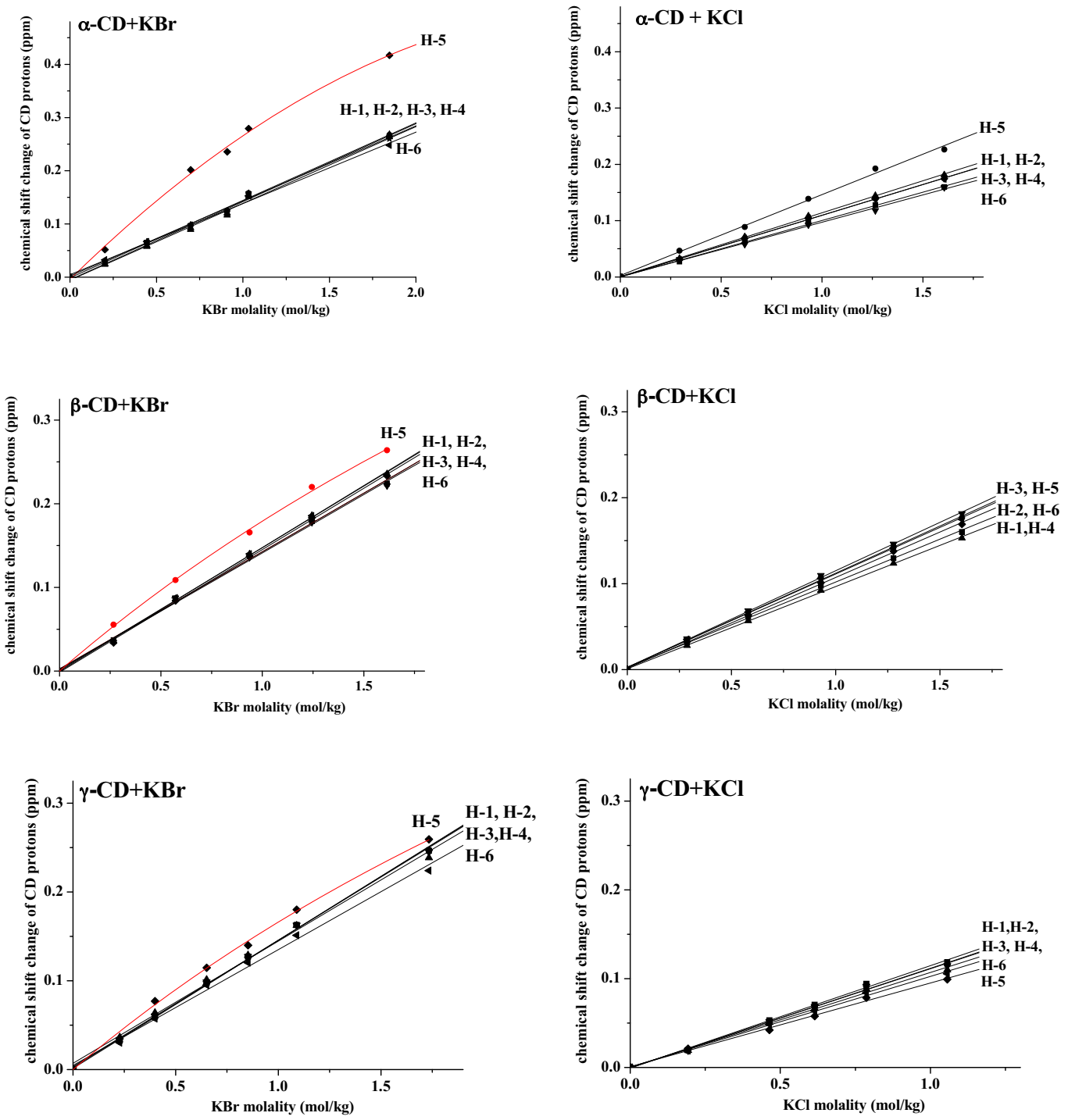

Fig. 4. Dependences of chemical shift changes of CD protons *versus* salt concentration at 298.15 K.

The performed ^{1}H NMR study confirmed that Br^- anions participate in the binding with CDs and compete with BA molecules for the placement inside the macrocyclic cavity. In spite of very weak binding between Br^- and CDs, this process, nevertheless, affects the proportion of CD/BA inclusion complexes. In support of this hypothesis, we calculated the equilibrium concentration of CD/BA complexes in the systems (CD + BA) and (CD + BA + KBr) taking into consideration one and two reactions, respectively. As an example, dependences of the content of α-CD/BA complexes on BA concentration in water and in 0.2 M solutions of KBr are shown in Fig. 5. As it can be seen from Fig. 5, presence of KBr in the solution results in a decrease of binding affinity of α-CD to BA, especially in the low concentration range. Thereby, complex formation of Br^- with CDs may be one of the reasons why binding constants for CD complexation with BA are appreciably decreased in the presence of KBr (Table 1 and Fig. 2).

As follows from Table 1 and Fig. 2, dihydrophosphate anions also substantially affect the binding of CDs with BA. However, effect of $H_2PO_4^-$ depends on the binding mode of BA with CD. For complex formation of α-CD with BA, the influence of $H_2PO_4^-$ is evident in decreasing K_{app}. On the contrary, an increase of K_{app} is observed for binding of BA with β-CD and γ-CD. It has been mentioned above that inclusion of BA in the α-CD cavity is shallow. It is accompanied by the location of a part of the aromatic ring near H-3 protons of

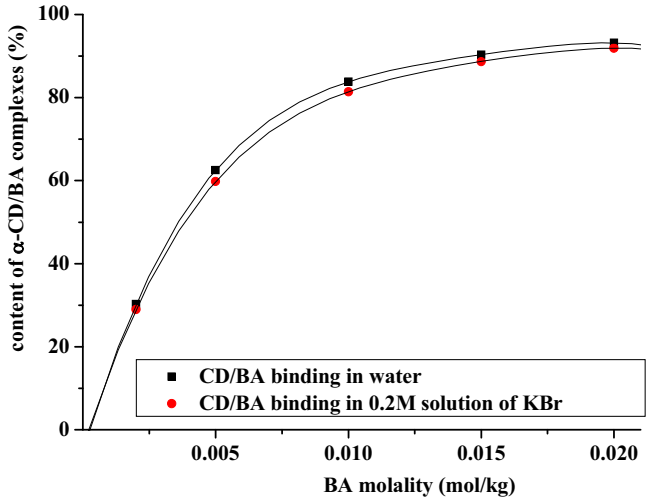

Fig. 5. Content of α-CD/BA complexes in water and 0.2 M solution of KBr *versus* BA concentration.

α-CD and the carboxylic acid group near H-5 protons. This binding mode is confirmed by the upfield shift of H-3 protons ($\Delta\delta < 0$) and downfield shift of H-5 protons ($\Delta\delta > 0$). Complex formation of BA with β-CD and γ-CD is characterized by the upfield shift of H-3 and H-5 protons and corresponds to deep insertion of BA molecule. In this case, the aromatic ring is completely placed inside the macrocyclic cavity and carboxylic acid group is located at the narrow rim of the CD molecule in the vicinity of primary OH-groups surrounding the cavity (Lu et al., 2000; Salvatierra et al., 1996). ^{1}H NMR spectra supporting the proposed binding modes are given in SI.

According to the solubility measurements (Fig. 1), KH_2PO_4 acts as salting-in agent and we might therefore expect it to reduce the BA activity coefficient and K_{app}. This phenomenon was observed for the binding of BA with α-CD (Table 1). However, more pronounced decrease of K_{app} compared to the predicted value was detected (Fig. 2). An opposite effect of KH_2PO_4 was revealed for the complexation of BA with β-CD and γ-CD (Fig. 2), since values of K_{app} increase instead of decrease. For explanation of the obtained results we assumed the possibility of specific interactions between $H_2PO_4^-$ and BA and took into account that $H_2PO_4^-$ anions are not included into CD cavity (Fig. 3).

To study the interactions of BA with dihydrophosphate we performed UV-spectroscopic measurements. Absorption spectra of BA in the presence of variable amounts of KH_2PO_4 (0–0.7 mol/kg) were recorded. For comparison, similar measurements were done with KCl, cations and anions of which are indifferent with respect to BA (Terekhova et al., 2010). Dependences of the absorbance on salt concentration are shown in Fig. 6. It is evident from Fig. 6 that addition of the first portion of KH_2PO_4 induces a sharp decrease of the absorbance, then the absorbance monotonically increases with the subsequent rise of KH_2PO_4 concentration. On the contrary, the absorbance of BA solution is slightly decreased with increasing KCl concentration, which means that no specific interactions in this system. Thus, the difference in the behavior of KH_2PO_4 and KCl is evident. First of all, the pronounced jump of the absorbance in the presence of KH_2PO_4 may refer to the change of the BA ionization state. To prove this fact we measured the pH of the solutions. Experimental pH values of BA solutions (2×10^{-4} mol/kg) without and with KH_2PO_4 (0.1 M) were 4.0 and 4.3, respectively. Further growth of KH_2PO_4 concentration up to 0.7 mol/kg resulted in negligible enhancement of pH. Taking into account the value of BA dissociation constant ($pK_a = 4.2$ (Chakravorty, Pal, & Lahiri, 1988)) one can conclude that the content of the ionized BA is higher at pH 4.3 than at 4.0. Thus, KH_2PO_4 shifts the equilibrium of BA dissociation, and the content of anionic species becomes appreciable (~55%). It means that in the presence of potassium dihydrophosphate CDs interact with both un-ionized and ionized forms of BA. Lower binding affinity of CDs to benzoate is well known (Simova & Schneider, 2000; Terekhova et al., 2010). Thus, decrease of K_{app} is logical, however it is observed only for α-CD binding with BA and does not explain the rise of K_{app} in case of complex formation with β-CD and γ-CD in KH_2PO_4 solution.

There are some instances in the literature which showed that increasing stability of CD/guest complexes in salt solutions can be attributed to ternary complex formation (Dey, Roberts, & Warner, 1998; Johnson & Bernard, 1996; Song & Bai, 2009). In ternary complex formation, the anion interacts with both host and guest and, therefore, acts as a bridge between these molecules (Johnson & Bernard, 1996). For example, it has been demonstrated by Dey et al. (1998) that the anion ClO_4^- interacts with the primary hydroxyl group(s) of β-CD and stabilizes the host-guest complex. Ternary complex formation was found to be predominant in the system (β-CD + tryptophan + CO_3^{2-}) (Song & Bai, 2009). Similarly, we propose the formation of ternary complexes β-CD/BA/$H_2PO_4^-$ and γ-CD/BA/$H_2PO_4^-$, in which dihydrophosphate anions simultaneously interact with CD and BA through specific ion-molecular interactions. This becomes possible due to the location of the BA carboxylic acid group in the vicinity of OH-groups surrounding the narrow rim of β-CD and γ-CD molecules. Ability of $H_2PO_4^-$ to hydrogen bonding with CD hydroxyls has been shown by Johnson and Bernard (1996). Additionally, the formation of strong hydrogen bonds between $H_2PO_4^-$ and 3,4-cis-dihydroxy tetrahydrofuran

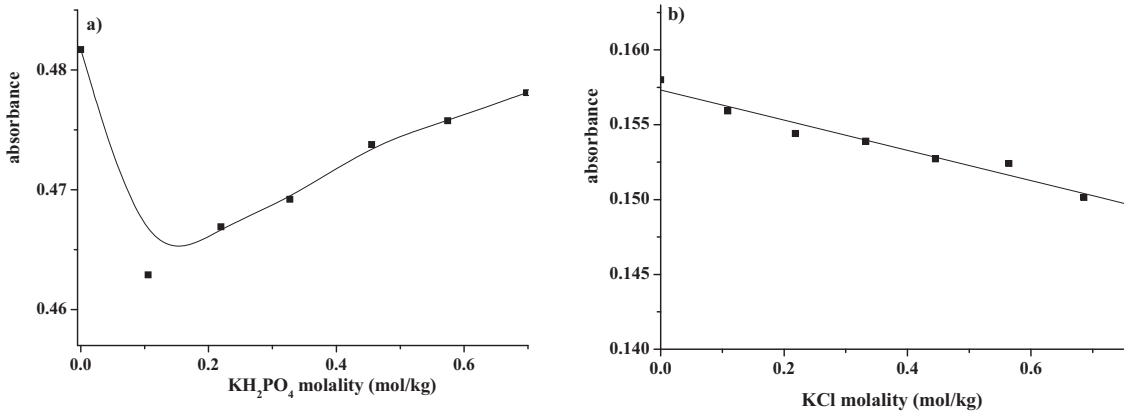

Fig. 6. Absorbance of BA solution *versus* the concentration of KH_2PO_4 (a) and KCl (b).

has been detected by Ha, Suleimenova, and Han (2001). Propensity of $H_2PO_4^-$ anions to interact with the un-ionized BA can be seen from Fig. 6. As it was discussed above, addition of KH_2PO_4 to an aqueous solution of BA results in an increase of pH and decrease of absorbance due to BA ionization. The consequent rise of KH_2PO_4 concentration resulted in insignificant change of pH and, therefore, the content of BA anions. In this case we expected that absorbance will be invariant. However, it was not true, and the absorbance was smoothly increased (Fig. 6). This probably occurs due to an existence of specific interactions between the BA molecule and the $H_2PO_4^-$ anion, which can be rationalized by evoking hydrogen bonding. Hydrogen binding can be also realized through water molecules, which can serve as a link between $H_2PO_4^-$ and organic reagent (Smolin, Lapshin, & Pankova, 2003).

Summarizing the results concerning the role of $H_2PO_4^-$ anion in the inclusion complex formation of BA with different CDs, we note that its influence depends on the CD structure and CD-BA binding mode. In case of deep insertion of BA molecule, when the carboxylic acid group is outside the macrocyclic cavity, the ternary complex formation takes place and promotes the enhancement of complexation efficiency.

4. Conclusions

The present experimental study revealed the effects of biologically active inorganic anions (Cl^-, Br^-, $H_2PO_4^-$ and SO_4^{2-}) in the inclusion complex formation of BA with α-CD, β-CD and γ-CD. It has been demonstrated that the change of the BA activity coefficient is only one factor affecting the CD-BA binding in the presence of KCl and K_2SO_4, while the additional ion-molecular interactions influence the complexation process in the presence of KBr and KH_2PO_4. Among the considered anions only Br^- anions are able to compete with BA molecules for penetration in the CD cavity and cause a decrease of the binding constants. The $H_2PO_4^-$ anions can specifically interact with organic reagents and their effect on the inclusion complex formation depends on CD-BA binding mode.

Acknowledgments

This work received financial support from Russian Foundation for Basic Research (project No 12-03-97516_r_center_a). We thank "The upper Volga region center of physicochemical research" for scientific equipment provided.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.04.057.

References

Bergeron, R. J., Channing, M. A., & McGovern, K. A. (1978). Dependence of cycloamylose-substrate binding on charge. *Journal of the American Chemical Society*, *100*, 2878–2883.

Brewster, M. E., & Loftsson, T. (2007). Cyclodextrins as pharmaceutical solubilizers. *Advanced Drug Delivery Reviews*, *59*, 645–666.

Chakravorty, S. K., Pal, A., & Lahiri, S. C. (1988). Thermodynamics for the ionization of benzoic acid in ethanol + water mixtures at 298.1 K. *Thermochimica Acta*, *124*, 43–52.

Del Valle, E. M. M. (2004). Cyclodextrins and their uses: A review. *Process Biochemistry*, *39*, 1033–1046.

Dey, J., Roberts, E. L., & Warner, I. M. (1998). Effect of sodium perchlorate on the binding of 2-(4′-aminophenyl)- and 2-(4′-(*NN*′-dimethylamino) phenyl)benzothiazole with α-cyclodextrin in aqueous solution. *Journal of Physical Chemistry A*, *102*, 301–305.

Fu, Y., Liu, L., & Guo, Q.-X. (2002). A theoretical study on the inclusion complexation of cyclodextrins with inorganic cations and anions. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *43*, 223–229.

Gelb, R. I., Schwartz, L. M., Radeos, M., & Laufer, D. A. (1983). Cycloamylose complexation of inorganic anions. *Journal of Physical Chemistry*, *87*, 3349–3354.

Ha, T. K., Suleimenova, O. M., & Han, M. J. (2001). A theoretical study of the anionic intermolecular hydrogen bonding between dihyroxy tetrahydrofuran and phosphate ions. *Journal Molecular Structure (Theochem)*, *574*, 75–83.

Johnson, M. D., & Bernard, J. G. (1996). Hydrogen bonding effects on the cyclodextrin encapsulation of transition metal complexes: 'Molecular snaps'. *Chemical Communications*, *2*, 185–186.

Kamiya, M., Mitsuhashi, S., Makino, M., & Yoshioka, H. (1992). Analysis of the induced rotational strength of mono- and disubstituted benzenes included in β-cyclodextrin. *Journal of Physical Chemistry*, *96*, 95–99.

Kano, K., Tanaka, N., Minamizono, H., & Kawakita, Y. (1996). Tetraarylporphyrins as probes for studying mechanism of inclusion-complex formation of cyclodextrins. Effect of microscopic environment on inclusion of ionic guests. *Chemistry Letters*, *25*, 925–926.

Lu, C.-S., Hu, C. J., Yu, Y., & Meng, Q.-J. (2000). The inclusion compounds of β-cyclodextrin with 4-substituted benzoic acid and benzaldehyde drugs studied by proton nuclear magnetic resonance spectroscopy. *Chemical and Pharmaceutical Bulletin*, *48*, 56–59.

Marcus, Y. (2012). Ion properties. In A. Ciferri, & A. Perico (Eds.), *Ionic interactions in natural and synthetic macromolecules* (pp. 3–33). Hoboken, NJ: John Wiley & Sons, Inc (Chapter 1)

Matsiu, Y., Ono, M., & Tokunaga, S. (1997). NMR spectroscopy of cyclodextrin-inorganic anion systems. *Bulletin of the Chemical Society of Japan*, *70*, 535–541.

Mochida, K., Kagita, K., Matsui, Y., & Date, Y. (1973). Effects of inorganic salts on the dissociation of a complex of β-cyclodextrin with an azo dye in an aqueous solution. *Bulletin of the Chemical Society of Japan*, *46*, 3703–3707.

Rohrbach, R. P., Rodriguez, L. J., Eyring, E. M., & Wojclk, J. F. (1977). An equilibrium and kinetic investigation of salt-cycloamylose complexes. *Journal of Physical Chemistry*, *81*, 944–948.

Romanova, A. O., Chibunova, E. S., Kumeev, R. S., Fedorov, M. V., & Terekhova, I. V. (2013). α-Cyclodextrin/aminobenzoic acid binding in salt solutions at different pH: Dependence on guest structure. *International Journal of Biological Macromolecules*, *57*, 255–258.

Salvatierra, D., Jaime, C., Virgili, A., & Sanchez-Ferrando, F. (1996). Determination of the inclusion geometry for the β-cyclodextrin/benzoic acid complex by NMR and molecular modeling. *Journal of Organic Chemistry*, *61*, 9578–9581.

Sanemasa, I., Fujiki, M., & Deguchi, T. (1988). A new method for determining cyclodextrin complex formation constants with electrolytes in aqueous medium. *Bulletin of the Chemical Society of Japan*, *61*, 2663–2665.

Santos, C. I. A. V., Ribeiro, A. C. F., Veríssimo, L. M. P., Lobo, V. M. M., & Esteso, M. A. (2013). Influence of potassium chloride on diffusion of 2-hydrohypropyl-β-cyclodextrin at T = 298.15 K and T = 310.15 K. *Journal of Chemical Thermodynamics*, *57*, 220–223.

Schneider, H.-J., Hacket, F., & Rüdiger, V. (1998). NMR studies of cyclodextrins and cyclodextrin complexes. *Chemical Reviews*, *98*, 1755–1785.

Shtenberg, A. J., & Ignat'ev, A. D. (1970). Toxicological evaluation of some combinations of food preservatives. *Food and Cosmetic Toxicology*, *8*, 369–380.

Siimer, E., & Kurvits, M. (1989). Calorimetric studies of benzoic acid-cyclodextrin inclusion complexes. *Thermochimica Acta*, *140*, 161–168.

Simova, S., & Schneider, H.-J. (2000). NMR analyses of cyclodextrin complexes with substituted benzoic acids and benzoate anions. *Journal of Chemical Society, Perkin Transactions 2*, *10*, 1717–1722.

Smolin, Yu. I., Lapshin, A. E., & Pankova, G. A. (2003). Crystal structure of di-(L-serine)phosphate monohydrate $[C_3O_3NH_7]_2H_3PO_4H_2O$. *Physics of the Solid State*, *45*, 1893–1897.

Song, L. X., & Bai, L. (2009). Old drugs, new tricks: The effect of molecule-ion interactions on the precipitation-dissolution equilibrium of lithium carbonate in aqueous solution and on the chiral recognition of cyclodextrins to D-,L-tryptophan. *Journal of Physical Chemistry B*, *113*, 11724–11731.

Spencer, J. N., He, Q., Ke, X., & Wu Zh Fetter, E. (1998). Complexation of inorganic anions and small organic molecules with alpha-cyclodextrin in water. *Journal of Solution Chemistry*, *27*, 1009–1019.

Strong, L. E., Neff, R. M., & Whitesel, I. (1989). Thermodynamics of dissolving and solvation processes for benzoic acid and the toluic acids in aqueous solution. *Journal of Solution Chemistry*, *18*, 101–114.

Taraszewska, J., & Wójcik, J. (1993). Complexation of inorganic anions by β-cyclodextrin studied by polarography and ^1H NMR. *Supramolecular Chemistry*, *2*, 337–343.

Terekhova, I. V. (2011). Comparative thermodynamic study on complex formation of native and hydroxypropylated cyclodextrins with benzoic acid. *Thermochimica Acta*, *526*, 118–121.

Terekhova, I. V., Chibunova, E. S., Kumeev, R. S., & Alper, G. A. (2013). Role of biologically active inorganic anions Cl^- and Br^- in inclusion complex formation of α-cyclodextrin with some aromatic carboxylic acids. *Chemical Physics Letters*, *557*, 134–139.

Terekhova, I. V., Romanova, A. O., Kumeev, R. S., & Fedorov, M. V. (2010). Selective Na^+/K^+ effects on the formation of α-cyclodextrin complexes with aromatic

carboxylic acids: Competition for the guest. *Journal of Physical Chemistry B*, *114*, 12607–12613.
Uekama, K. (2002). Recent aspects of pharmaceutical application of cyclodextrins. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *44*, 3–7.
Wojcik, J. F., & Rohrbach, R. P. (1975). Small anion binding to cycloamylose. Equilibrium constants. *Journal of Physical Chemistry*, *79*, 2251–2253.
Yi, Z., Zhao, C., Huang, Z., Chen, H., & Yu, J. (1999). Investigation of buffer-cyclodextrin systems. *Physical Chemistry Chemical Physics*, *1*, 441–444.
Yurquina, A., Manzur, M. E., Brito, P., Manzo, R., & Molina, M. A. A. (2003). Solubility and dielectric properties of benzoic acid in a binary solvent: Water–ethylene glycol. *Journal of Molecular Liquids*, *108*, 119–133.
Zhang, X. J., Qin, H. W., Su, L. M., Qin, W. C., Zou, M. Y., Sheng, L. X., et al. (2010). Interspecies correlations of toxicity to eight aquatic organisms: Theoretical considerations. *Science of the Total Environment*, *408*, 4549–4555.