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ORIGINAL ARTICLE

# $^1$H NMR and calorimetric study on binding ability of cyclodextrins to isomeric pyridinecarboxylic acids in aqueous solution

**Irina V. Terekhova · Roman S. Kumeev · Gennady A. Alper**

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**Abstract** Complex formation of α- and β-cyclodextrins with isomeric pyridinecarboxylic acids (picolinic, nicotinic and isonicotinic acids) in water were studied by calorimetry and $^1$H NMR at 298.15 K. The obtained results revealed the weak 1:1 complex formation governed by the cavity dimensions and position of the carboxylic group in the pyridine ring. It was found that selective inclusion complex formation of α- and β-cyclodextrins with nicotinic and isonicotinic acids takes place. In the case of picolinic acid, the considerable role of external interactions and formation of less stable complexes were detected. The location of the carboxylic group in the *meta*-position of pyridine ring is more favorable for the effective binding. The pyridinecarboxylic acids are shallow inserted into α-cyclodextrin cavity possessing the smaller diameter, while they are deeply included into β-cyclodextrin cavity. Thermodynamic parameters of complex formation (K, $\Delta_cG^0$, $\Delta_cH^0$ and $\Delta_cS^0$) were calculated and discussed in terms of influence of reagents structure.

**Keywords** Cyclodextrin · Pyridinecarboxylic acid · Inclusion complex formation · NMR · Calorimetry · Thermodynamics

## Introduction

Cyclodextrins (CDs, Fig. 1) are well-known macrocyclic receptors in supramolecular chemistry, which can form inclusion complexes (or host-guest complexes) with a wide variety of organic compounds [1–3]. Native α-, β- and γ-CD consist of six, seven and eight glucose units, respectively, and differ from each other by the cavity diameter. Inclusion complexes of CDs received the numerous practical applications in pharmaceutical, food, cosmetic, textile industries and separation technologies [4–8]. It has been also detected that inclusion complex formation with CDs can significantly improve the physicochemical properties of biologically active compounds (e.g. stability, solubility, test), prolong their bioactivity and remove unwanted side effects [4–7]. Moreover, CDs can be used as drug delivery systems [9]. Thus, the investigation of CD's binding ability to biomolecules is of practical importance.

Pyridinecarboxylic acids (PA, Fig. 1) and their derivatives present in many natural products and possess the physiological activity. In particular, nicotinic acid (pyridine-3-carboxylic acid, vitamin $B_5$) is necessary for normal functioning of living organism and it is widely used as cholesterol-reducing and antipellagra drug [10]. Picolinic acid (pyridine-2-carboxylic acid) is considered as the body's natural chelating agent and can be found in some dietary supplements [11]. Moreover, isonicotinic (pyridine-4-carboxylic acid) and picolinic acids are employed as intermediates to produce pharmaceuticals [12]. To the best of our knowledge, complex formation of CDs with pyridinecarboxylic acids was not previously studied in detail. Only a few publications concerning study on interactions of CDs with nicotinic acid and isonicotinic acid derivatives were found in literature [13–15]. As it was obtained by microcalorimetry, ionized nicotinic acid did not give a measurable heat of reaction with CDs at pH = 7.0 [13]. However, this result can not be considered as the evidence of absence of complex formation between CD and nicotinic acid. On the contrary, inclusion complex formation was detected in the freeze-dried products obtained from aqueous solutions of nicotinic acid and β-CD [14].

I. V. Terekhova (✉) · R. S. Kumeev · G. A. Alper
Institute of Solution Chemistry, Russian Academy of Sciences, 153045 Ivanovo, Russian Federation
e-mail: ivt@isc-ras.ru

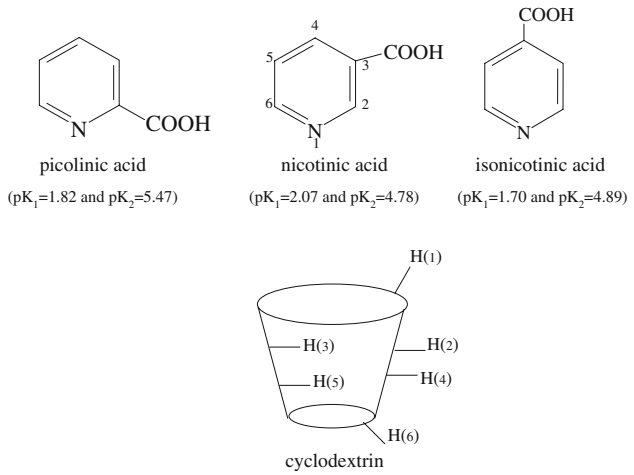


**Fig. 1** Schematic presentation of objects under study

This work is a continuation of previously started investigation devoted to study on complex formation of CDs with isomeric pyridinecarboxylic acids. In particular, inclusion complex formation of nicotinic acid with native and hydroxypropylated α-CD was examined in detail using calorimetry [16, 19], $^1$H NMR spectroscopy [17, 18], densimetry [19] and capillary electrophoresis [20]. Herein, the $^1$H NMR spectroscopic and calorimetric investigation of interactions of α- and *β*-CD with picolinic and isonicotinic acids in water at 298.15 K was continued. Some interest was directed to the analysis of the influence of the position of carboxylic group in the pyridine ring and the size of macrocyclic cavity on the binding mode and thermodynamic parameters of complex formation.

## Experimental

### Materials

Commercial available picolinic acid (Acros Organics), nicotinic acid (MP Biomedicals), isonicotinic acid (MP Biomedicals), α-CD (Fluka), *β*-CD (Fluka) were used as received. CDs were stable crystallohydrates, the water content in which was taken into account during preparation of solutions and calculation of their concentrations. All solutions were prepared by weight.

### $^1$H NMR study

$^1$H NMR spectra were recorded on Bruker AC-200 spectrometer operating at 200 MHz. Temperature of 298.15 K was held constant (±0.10 K) using a Bruker BVT-3000 temperature control system. All chemical shifts were measured in the deuterated water (99.9% isotopic purity) relative to external reference cyclohexane.

### Calorimetry

Home-made calorimeter of solution was used to measure the thermal effects of dissolution. The detailed description of the calorimetric installation was given elsewhere [16]. The crystalline samples of CDs of constant mass were dissolved in pure water and in aqueous solutions of pyridinecarboxylic acids of variable concentration at 298.15 K. The error of the thermal effects measurements was not >0.5%. To calculate the unknown parameters of complex formation, K (stability constant) and $\Delta_c H^0$ (enthalpy of complex formation), the experimental data were fitted to a theoretical titration curve using computer program "HEAT" [21]. Computer program "HEAT" allows to take into account the possible processes of pyridinecarboxylic acid dissociation, thermodynamic parameters of which were taken from literature [22, 23].

## Results and discussion

Pyridinecarboxylic acids under consideration are amphiphilic compounds, which predominantly exist as zwitterions in aqueous medium and dissociate according to Equilibria (1) and (2):

$$AH_2^+ \overset{k_1}{\longleftrightarrow} AH^{\pm} + H^+ \quad (1)$$

$$AH^{\pm} \overset{k_2}{\longleftrightarrow} A^- + H^+ \quad (2)$$

The dissociation constant values reported elsewhere [22, 23] were used for calculation of the distribution of the ionized forms of pyridinecarboxylic acids (anoin, cation and zwitterion) depending on pH. These dependences are presented in Fig. 2. The pH of solutions was controlled during all measurements and it was varied from 3.3 to 3.5 for nicotinic acid, from 3.1 to 3.4 for picolinic acid and from 3.4 to 3.5 for isonicotinic acid. The observed pH range corresponded to existence domain of zwitterions ($\alpha \geq 98\%$). It should be also noted that addition of CDs to the pyridinecarboxylic acid solutions induced unimportant pH shifts laying within the error limits. It indicates that CDs bind zwitterions and their interactions with cationic and anionic species can be neglected.

$^1$H NMR spectroscopy is a powerful method for elucidation of the binding mode, stability and stoichiometry of the complexes formed. It is well established [24], that $^1$H NMR spectrum of CD in $D_2O$ consists of the signals from six protons. Protons H(1), H(2), H(4) and H(6) are located on the external surface of the molecule, whereas H(3) and H(5) protons are placed in the interior of the cavity at the wide and narrow rims, respectively (Fig. 1). Only H(3) and H(5) protons are sensitive to penetration of the guest molecule inside the host cavity and, therefore, they are

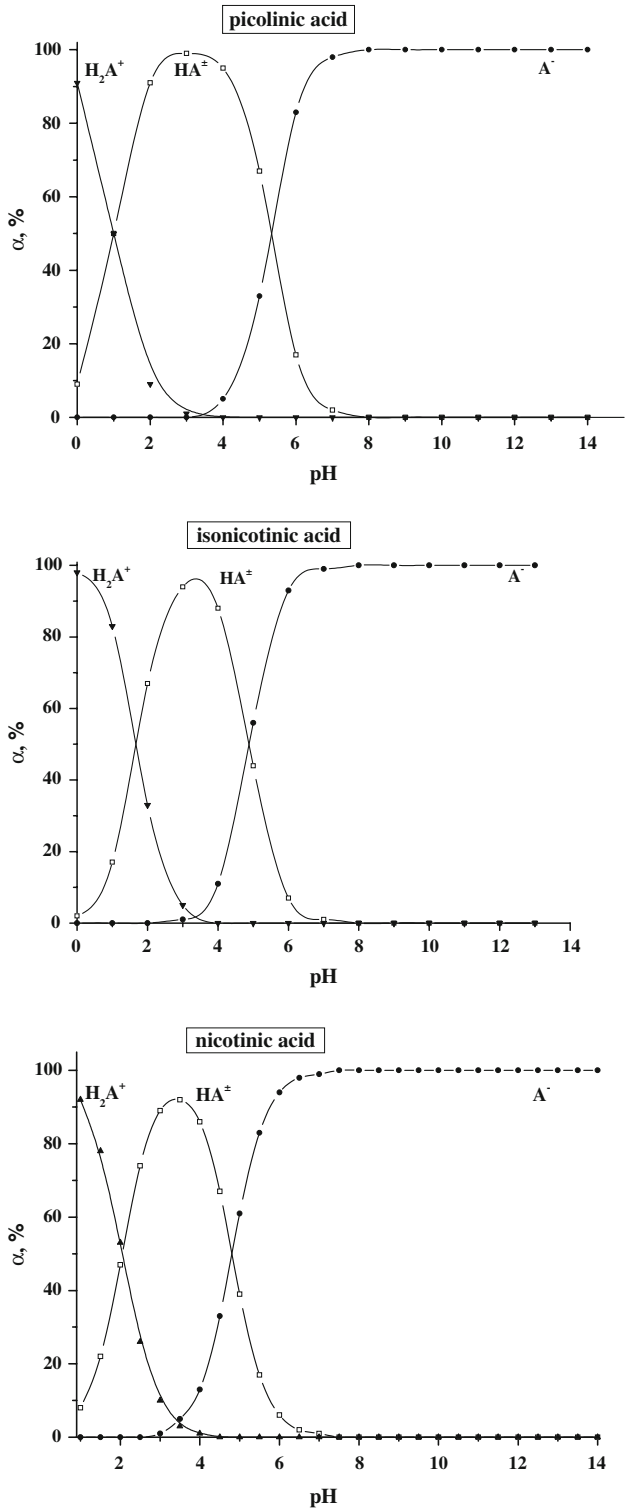


**Fig. 2** Distribution of ionized forms of pyridinecarboxilic acids in aqueous solution depending on pH (T = 298.15 K)

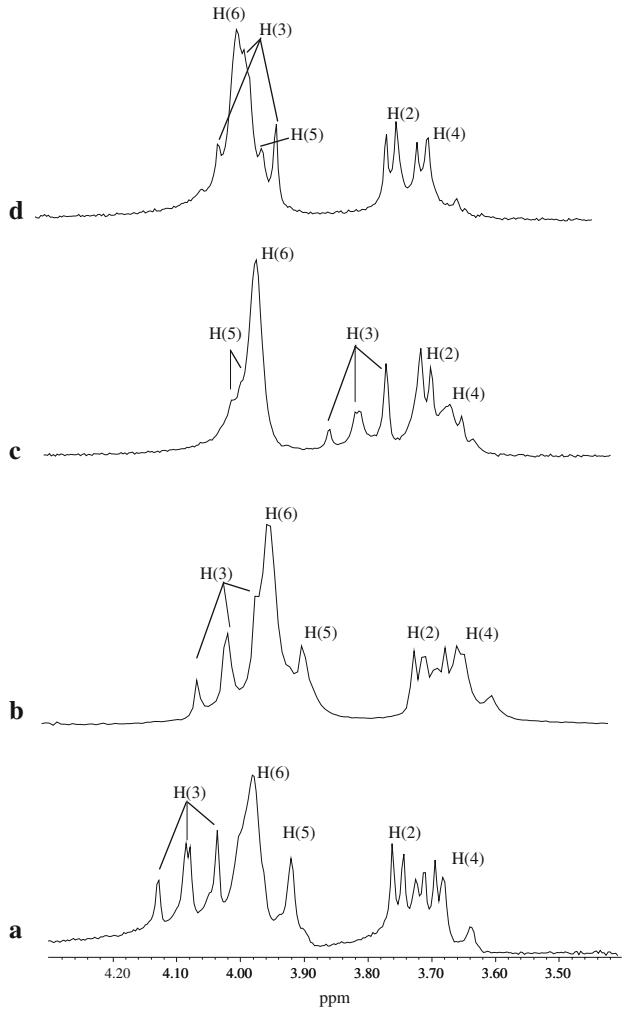


**Fig. 3** Partial $^1$H NMR spectra (200 MHz, 298.15 K) of α-CD (0.005 mol kg$^{-1}$) alone (**a**) and in the presence of pyridinecarboxylic acids (**b**) picolinic acid (0.25 mol kg$^{-1}$), (**c**) nicotinic acid (0.06 mol kg$^{-1}$), (**d**) isonicotinic acid (0.07 mol kg$^{-1}$)

responsible for the inclusion phenomenon. Figs. 3 and 4 contain the partial $^1$H NMR spectra of α- and *β*-CD, respectively, in the presence of the excess amounts of pyridinecarboxylic acids. Comparison of the plots indicates that addition of picolinic acid to the solutions of α- and *β*-CD results in the sizeable upfield shifts of the external and internal CD protons. This fact reveals the formation of both inclusion and external complexes. External complex formation of CDs is not so typical, however, in some cases it can take place. For example, the external binding was found for complex formation of *β*-CD with *tert*-butyl ketones [25]. On the contrary, the presence of nicotinic and isonicotinic acids induces the measurable upfield shits of the internal protons H(3) and H(5), denoting the formation of true inclusion complexes.

For calculation of the stability constants of the complexes the chemical shift changes (Δ*δ*) of CD protons were determined at constant concentration of CD (0.005 mol kg$^{-1}$) and variable concentration of picolinic acid (0–1.0 mol kg$^{-1}$), nicotinic acid (0–0.12 mol kg$^{-1}$) and isonicotinic

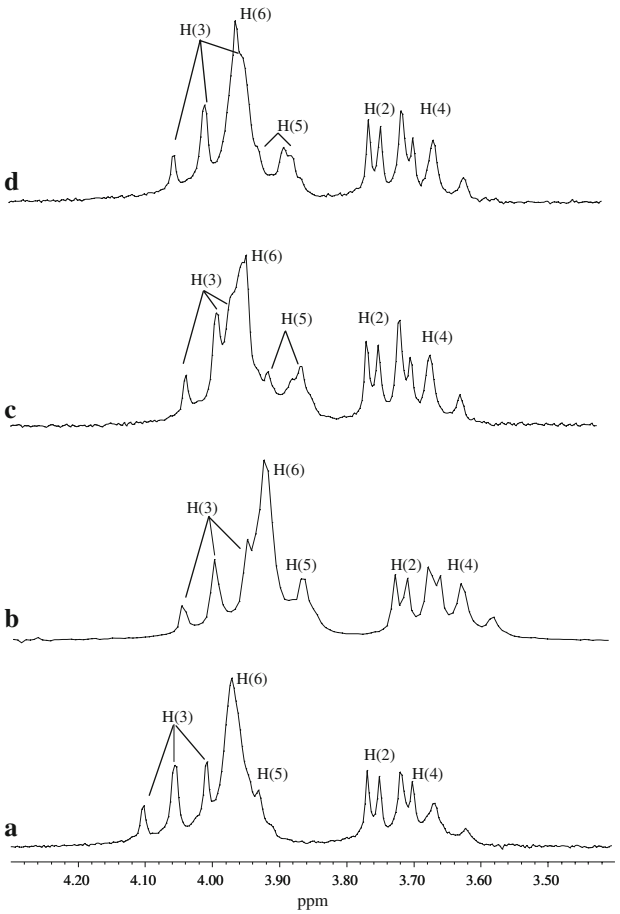


**Fig. 4** Partial $^1$H NMR spectra (200 MHz, 298.15 K) of $\beta$-CD (0.005 mol kg$^{-1}$) alone (**a**) and in the presence of pyridinecarboxylic acids (**b**) picolinic acid (0.25 mol kg$^{-1}$), (**c**) nicotinic acid (0.06 mol kg$^{-1}$), (**d**) isonicotinic acid (0.07 mol kg$^{-1}$)

acid (0–0.09 mol kg$^{-1}$). As examples, some concentration dependences of $\Delta\delta$ are shown in Fig. 5. The character of $\Delta\delta$ deviation with increasing concentration of pyridinecarboxylic acids points out the weak binding in all systems under study. To estimate the stoichiometry of the complexes the Benesi-Hildebrand method was used [26]. According to this method [26, 27], the concentration dependences of $\Delta\delta$ (Fig. 5) were converted in the double reciprocal plots ($1/\Delta\delta = f(1/C)$), the linear character of which testifies the 1:1 binding (Fig. 6). Moreover, the 1:1 composition of complexes of α-CD with nicotinic acid was obtained by continuous variation method in our previous publications [17, 18]. Thus, the binding of CDs with pyridinecarboxylic acids is described by the Equation:

$$\mathrm{CD} + \mathrm{PA} \rightarrow \mathrm{CD} \cdot \mathrm{PA} \tag{3}$$

The observable chemical shift ($\delta$) of CD protons is determined by the following way:

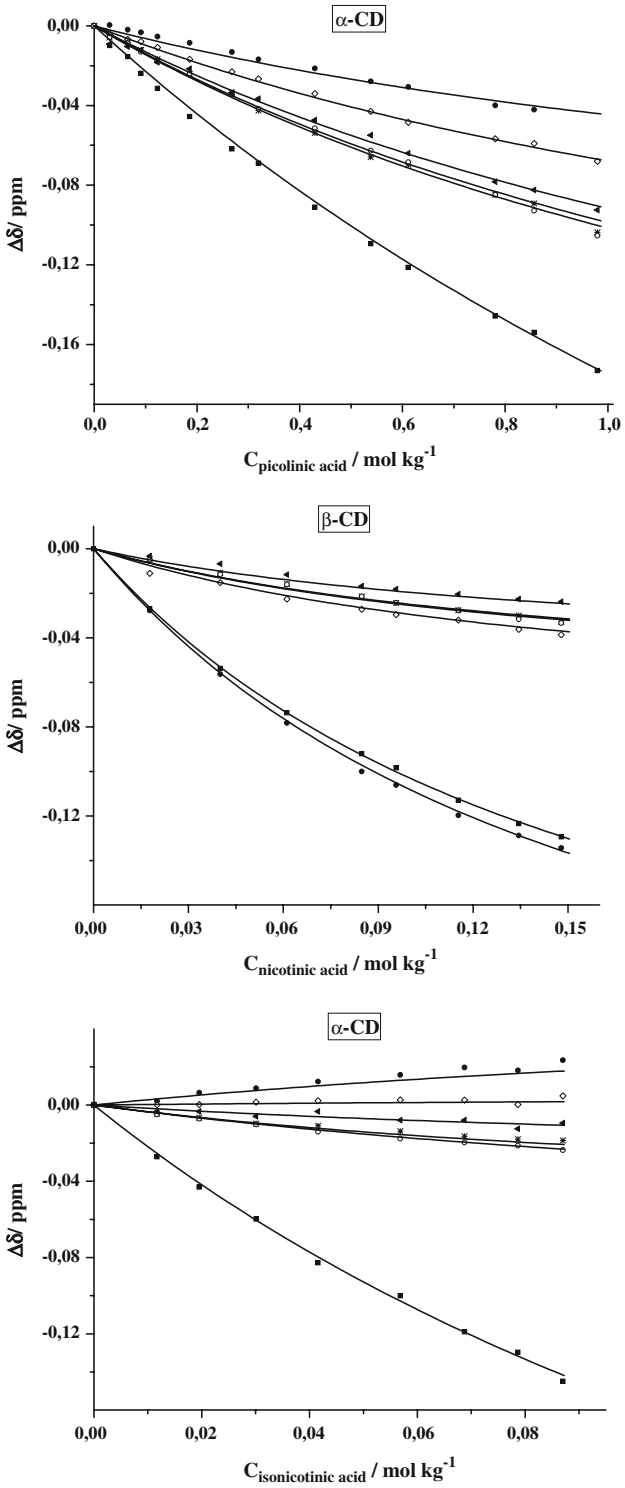


**Fig. 5** Chemical shift changes of CD protons versus the pyridinecarboxylic acid concentration (*, H(1); ◇, H(2); ◆, H(3); ●, H(4); ○, H(5); ▲, H(6))

$$\delta = \alpha \cdot \delta_f + (1 - \alpha) \cdot \delta_c \tag{4}$$

where $\alpha$ is the fraction of free CD; $\delta_f$ and $\delta_c$ are the chemical shifts of free and complexed CD, respectively.

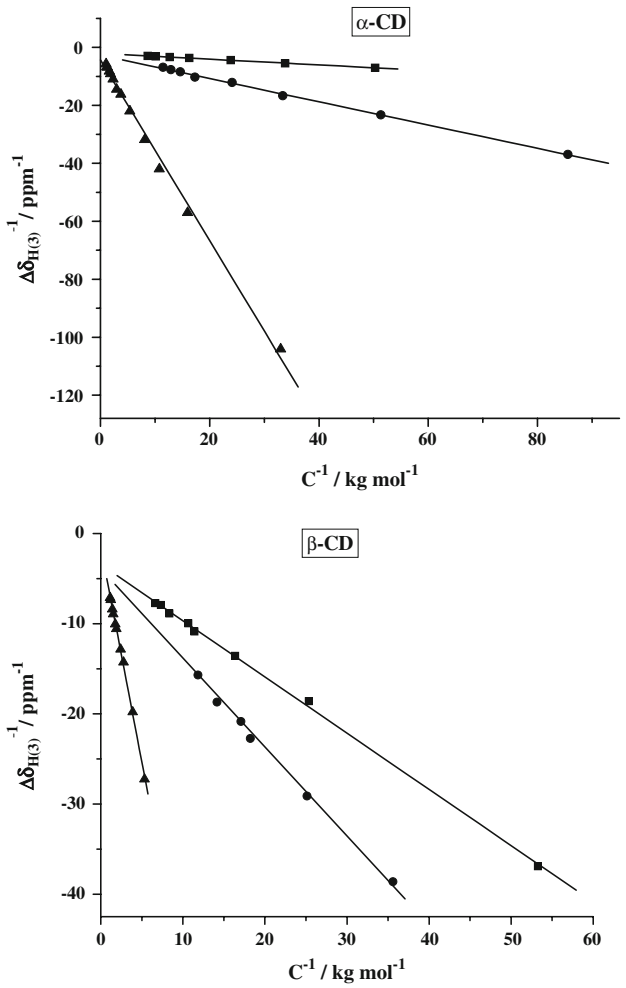


**Fig. 6** Double reciprocal plots for complex formation of CDs with pyridinecarboxylic acids in water at 298.15 K (▲, nicotinic acid; ●, isonicotinic acid; ■, picolinic acid)

Letting $\Delta\delta = \delta - \delta_f$ and $\Delta\delta_c = \delta_c - \delta_f$ one can obtain Equation for equilibrium constant:

$$K = C_{CD} \cdot \Delta\delta/(\Delta\delta_c \cdot (C_{CD} - C_{CD} \cdot \Delta\delta/\Delta\delta_c) \cdot (C_{PA} - C_{CD} \cdot \Delta\delta/\Delta\delta_c)) \quad (5)$$

where $C_{CD}$ and $C_{PA}$ are the initial concentrations of CD and pyridinecarboxylic acid, respectively. Analytical solution of Eq. 5 by non-linear regression analysis gives $\Delta\delta_c$ and K, the values of which are listed in Table 1.

Analysis of $\Delta\delta_c$ values reported in Table 1 shows that the signals of H(3) and/or H(5) protons of α- and β-CD are significantly shifted upon complex formation with all pyridinecarboxylic acids under study. The minor chemical shift changes of the outer CD protons were detected in case of binding with nicotinic and isonicotinic acids. It means that selective inclusion complex formation occurs in these systems. On the contrary, the unexpectedly large upfield shifts of H(1), H(2), H(4) and H(6) protons of both CDs were obtained for complex formation with picolinic acid. This fact indicates that external interactions together with the inclusion complex formation take place in this system.

For α-CD complexation, the largest upfield chemical shift changes were obtained only for H(3) protons, which are located in the shielding area of the aromatic ring of pyridinecarboxylic acids. These upfield shifts are due to ring current effects of the included aromatic guest molecule [28, 29]. Considerable shifting of signals from H(3) protons can be explained by the shallow insertion of pyridinecarboxylic acids into α-CD cavity in the direction from the wider rim. This fact is clearly confirmed by the absence of interactions with the external proton H(6) which is located near the narrow rim of the torus and less unaffected by the guest penetration. The proposed binding mode of α-CD with pyridinecarboxylic acid can be caused by the smaller diameter of its macrocyclic cavity.

As it is evident from Table 1, the H(3) and H(5) protons of β-CD exhibit large and approximately identical chemical shift changes. Thus, β-CD possessing the larger cavity diameter is able to include pyridinecarboxylic acid molecule more deeply. Moreover, the formation of two kinds of 1:1 inclusion complexes, in which pyridinecarboxylic acid is inserted from the both narrow and wide rims, can be assumed. In particular, the inclusion of pyridinecarboxylic acid from the wider rim can be proved by the measurable $\Delta\delta_c$ values for H(6) protons.

Insertion of pyridinecarboxylic acids into CD cavity occurs in accordance with the principle of geometric complementarity. Therefore, comparison of the geometric parameters of gust and host can be useful. It is well established, that cavity diameter of α- and β-CD is 4.7–5.3 and 6.0–6.5 Å, respectively [2, 4]. The cavity depth of both CDs is 7.9 Å [2, 4]. Geometric dimensions of pyridinecarboxylic acids were calculated using HyperChem program (v. 7.01). They were 5.8 × 4.0, 6.8 × 4.4 and 6.8 × 4.3 Å$^2$ for isonicotinic, nicotinic and picolinic acids, respectively. It is not difficult to see that α-CD cavity is small for placement of whole molecule of pyridinecarboxylic acid. Shallow and snugly fitting can be observed in this case. On the contrary, the β-CD cavity is more suitable for full incorporation of pyridinecarboxylic acids. These results are in accordance with the proposed binding modes based on the $^1$H NMR data.

Calorimetry is the method that directly allows to obtain the thermodynamics of complex formation (stability constants, Gibbs energy, enthalpy and entropy). Isotherms of binding are presented in Fig. 7. Thermodynamic parameters of complex formation of α- and β-CD with pyridinecarboxylic acids are summarized in Table 2. These values show that binding of α- and β-CD with considered acids is different from the thermodynamic point of view. The α-CD complexation is characterized by more negative enthalpy and entropy values (enthalpy driven). With the exception of systems with picolinic acid, β-CD complex

**Table 1** Stability constants of the complexes and the chemical shift changes of cyclodextrin protons induced by 100% complexation with pyridinecarboxylic acids in water at 298.15 K.

| Complex | K (kg mol$^{-1}$) | $\Delta\delta_c$ (ppm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | H(1) | H(2) | H(3) | H(4) | H(5) | H(6) |
| α-CD/picolinic acid | ≤1 | −0.39 ± 0.01 | −0.39 ± 0.01 | −0.66 ± 0.01 | −0.37 ± 0.01 | −0.19 ± 0.01 | −0.27 ± 0.01 |
| β-CD/picolinic acid | 1.0 ± 0.1 | −0.37 ± 0.01 | −0.35 ± 0.01 | −0.57 ± 0.03 | −0.35 ± 0.01 | −0.57 ± 0.01 | −0.38 ± 0.01 |
| α-CD/isonicotinic acid | 5.3 ± 0.1 | −0.07 ± 0.01 | −0.08 ± 0.01 | −0.48 ± 0.08 | −0.04 ± 0.01 | 0.09 ± 0.01 | <0.01 |
| β-CD/isonicotinic acid | 3.3 ± 0.1 | −0.07 ± 0.01 | −0.07 ± 0.01 | −0.29 ± 0.01 | −0.05 ± 0.01 | −0.31 ± 0.01 | −0.14 ± 0.01 |
| α-CD/nicotinic acid | 23.4 ± 0.8 | −0.05 ± 0.01 | −0.07 ± 0.01 | −0.42 ± 0.01 | −0.01 ± 0.01 | 0.11 ± 0.01 | −0.01 ± 0.01 |
| β-CD/nicotinic acid | 6.5 ± 0.5 | −0.06 ± 0.01 | −0.06 ± 0.01 | −0.27 ± 0.01 | −0.05 ± 0.01 | −0.26 ± 0.01 | −0.07 ± 0.01 |

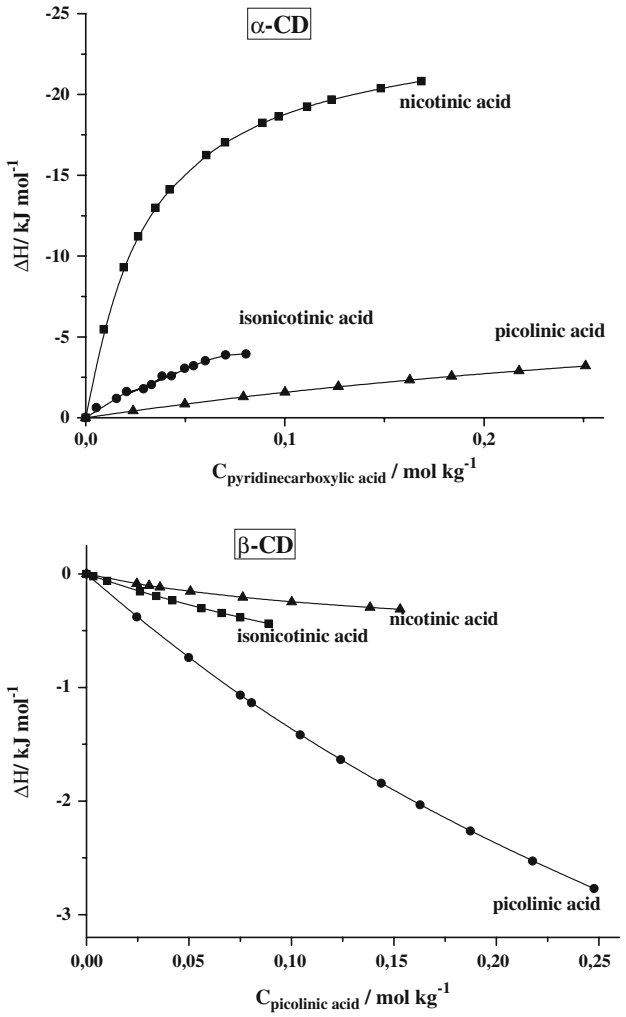


**Fig. 7** Isotherms of binding of cyclodextrins with pyridinecarboxylic acids in water (T = 298.15 K)

formation is accompanied by small negative enthalpies and positive entropies (enthalpy–entropy driven). This marked difference can be caused by the different cavity size and binding mode.

As it is well known, thermodynamic parameters of complex formation contain the contributions from the following main processes: dehydration of the reagents, binding of reagents through noncovalent interactions (van der Waals, electrostatic, hydrophobic interactions, hydrogen bonding) and hydration of the complex. We tried to explain the obtained difference in the thermodynamics of complex formation of CDs taking into account (1) the contributions from all these processes, (2) the binding mode proposed on the basis of $^1$H NMR measurements and (3) the revealed orders of increase in stability constants and exothermicity of binding:

$$\text{for } \alpha\text{−CD} \quad K: \quad \text{nicotinic acid} > \text{isonicotinic acid} > \text{picolinic acid}$$

$$-\Delta_c H^0: \quad \text{nicotinic acid} > \text{isonicotinic acid} > \text{picolinic acid}$$

$$\text{for } \beta\text{−CD} \quad K: \quad \text{nicotinic acid} > \text{isonicotinic acid} > \text{picolinic acid}$$

$$-\Delta_c H^0: \quad \text{picolinic acid} > \text{isonicotinic acid} > \text{nicotinic acid}$$

According to $^1$H NMR data, the shallow insertion of pyridinecarboxylic acids into smaller α-CD cavity takes place. In this case the tight fit of gust molecule into the host cavity is realized and accompanied by the partial dehydration. The attractive van der Waals interactions together with the possible electrostatic interactions and hydrogen bonding are responsible for the high exothermicity of the binding. These interactions are the driving force of complexation process and the main contribution to the stability of α-CD/pyridinecarboxylic acid complexes. However, despite the highly exothermic binding, the complexes possess very low stability. It is the result of compensation of the enthalpic contribution by the high and negative entropic contribution caused by the restriction of the conformational mobility of the reagents upon complex formation. The conformational restriction can arise from hydrogen bonding as well as van der Waals and possible electrostatic interactions.

The values of $\Delta_c H^0$ and $\Delta_c S^0$ are considerably higher for β-CD complex formation with nicotinic and isonicotinic

**Table 2** Thermodynamic parameters of complex formation of CDs with pyridinecarboxylic acids in water at 298.15 K

| Complex | K (kg mol$^{-1}$) | $\Delta_cG^0$ (kJ mol$^{-1}$) | $\Delta_cH^0$ (kJ mol$^{-1}$) | $T\Delta_cS^0$ (kJ mol$^{-1}$) |
|---|---|---|---|---|
| α-CD/picolinic acid | 1.8 ± 0.3 | −1.5 | −12.1 ± 0.6 | −10.6 |
| β-CD/picolinic acid | 1.8 ± 0.3 | −1.5 | −10.8 ± 0.5 | −9.3 |
| α-CD/isonicotinic acid | 7 ± 2 | −4.8 | −13.4 ± 0.4 | −8.6 |
| β-CD/isonicotinic acid | 3.3 ± 0.1[a] | −3.0 | −1.9 ± 0.2 | 1.1 |
| α-CD/nicotinic acid [16] | 33 ± 5 | −8.7 | −26.4 ± 0.3 | −17.7 |
| β-CD/nicotinic acid | 6.5 ± 0.5[a] | −4.6 | −0.6 ± 0.1 | 4.0 |

[a] For systems with weak complex formation and very low thermal effects stability constants obtained from $^1$H NMR measurements were used for calculation of enthalpy values

acids. The detected by $^1$H NMR technique deeper insertion of pyridinecarboxylic acids into β-CD cavity results in more intense dehydration of solutes. The dehydration and possible hydrophobic interactions between aromatic ring and cavity walls are predominant in this case. These processes characterized by the endothermic effect result in decrease of the $\Delta_cH^0$ values obtained for complex formation of β-CD with nicotinic and isonicotinic acids. Therefore, the enthalpy contribution to the free energy and complex stability is not prevalent in the case of β-CD complex formation. In contrast to enthalpy, $\Delta_cS^0$ values are favorable for binding of β-CD with nicotinic and isonicotinic acids. The main sours of observed positive $\Delta_cS^0$ values can be (a) probable hydrophobic interactions [3, 30, 31]; (b) release of water molecules from the hydration shells into the bulk water [32, 33]; (c) variety of complex configurations [34]. Thus, the structural rearrangement in these systems plays an important role and lead to an increase in the number of available microstates [34].

It is interesting to analyze the position of the carboxylic group on the thermodynamic parameters of complex formation. The observable orders of increase of complex stability and exothermicity of binding do not follow to the sequential moving of the carboxylic group from the nitrogen of the aromatic ring. It seems that placement of the carboxylic group in the *meta*-position is structurally favorable for the binding with CDs and formation of more stable inclusion complexes. In the case of picolinic acid, stability constants are very low, however, the binding occurs, according to the data reported in Tables 1 and 2. The size of the CD cavity has no essential influence on the thermodynamics of complex formation with picolinic acid. In addition, the unexpectedly large upfield shifts of the outer protons of CDs were obtained for this system (Table 1). These facts points out the important role of external interactions in the complexation of picolinic acid by CDs.

## Conclusion

On the basis of the obtained results, the possible structures of CD inclusion complexes with pyridinecarboxylic acids were proposed. Pyridinecarboxylic acids are shallow inserted in the α-CD cavity through its wider rim. The relatively tight fit between pyridinecarboxylic acid and α-CD cavity is realized via attractive interactions, which are accompanied by negative enthalpy and entropy changes. In the case of β-CD complex formation, the deeper inclusion of pyridinecarboxylic acids accompanied by more intensive dehydration was observed. As was found, complex formation of pyridinecarboxylic acids with α- and β-CD is affected by the macrocyclic cavity dimensions and position of the carboxylic group in the pyridine ring.

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