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Complexation of niflumic acid with native and hydroxypropylated α- and β-cyclodextrins in aqueous solution

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Interactions between niflumic acid and native and hydroxypropylated α- and β-cyclodextrins (CDs) were investigated by ^{1}H NMR, UV-vis spectroscopy, densimetry, and calorimetry at pH = 7.4 (phosphate buffer) and T = 298.15 K. Thermodynamic parameters of 1:1 complex formation were calculated and discussed in terms of influence of cavity size and availability of hydroxypropyl substituents on the complex stability. The ^{1}H NMR data indicated the inclusion of niflumic acid into macrocyclic cavity of all CDs under study. It was found that both phenyl and pyridine rings of niflumic acid molecule can be included in the cyclodextrin cavity. The co-existence of two different kinds of 1:1 inclusion complexes in the solution was suggested. In spite of the fact that binding of niflumic acid with α-cyclodextrin is more enthalpically favorable, stability of the inclusion complexes is very low due to the enthalpy–entropy compensation effect. Complex formation of β-CDs with niflumic acid is characterized by the higher enthalpy and entropy changes caused by more intense dehydration. Introduction of hydroxypropyl groups in the cyclodextrin molecule was found to promote the binding with niflumic acid. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: inclusion complex formation; cyclodextrin; niflumic acid; thermodynamics

INTRODUCTION

Niflumic acid (2-[3-(trifluoromethyl)anilino]nicotinic acid, NA, Fig. 1) belongs to a class of nonsteroidal anti-inflammatory drugs and acts by inhibiting isoforms of cyclo-axygenase. It is also a noncompetitive inhibitor of chloride exchange. Niflumic acid has an activity to treat inflammatory rheumatoid diseases and relieve acute pains. It is used during the period of pains and after surgery and fever. It is obvious that gastrointestinal absorption and therapeutic action of drugs depend on its solubility. Niflumic acid is a poorly water-soluble medicine, therefore, its low aqueous solubility can reduce activity and restrict practical applications. To increase the aqueous solubility and bioavailability of niflumic acid as well as to eliminate its unwanted side effects the encapsulation by cyclodextrins (CDs) can be proposed and used.[1–3] CDs are the cyclic oligosaccharides obtained by the enzymatic degradation of starch, therefore, they are nontoxic and safe for human health.[4–6] The ability of CDs to form inclusion complexes (or host–guest complexes) with a wide variety of organic compounds is well known[4,5,7] and determines the numerous practical applications of CDs as solubilizing, stabilizing, and encapsulating agents as well as drug delivery systems.[1–3,8] The inclusion complex formation with CDs can lead to changes in the physicochemical and biological properties of guest molecules.

To improve the aqueous solubility of niflumic acid, to prolong its therapeutic action, and to reduce unwanted side effects the complexation by CDs can be applied. Thus, the aim of our work is to reveal the ability of CDs to form inclusion complexes with niflumic acid in aqueous solution. This current research is a continuation of our previous investigation devoted to study on complex formation of niflumic acid with native and modified β-CDs.[9] Data on complex formation of niflumic acid with β-CDs were also reported in several publications.[10–13] In particular, Gezawi *et al.*[10] studied the complex formation of niflumic acid with β- and γ-CDs in aqueous solutions (pH 6.0 and 7.0) at 298.15 K by microcalorimetry and HPLC. It was shown that the cavity of β-CD molecule is more suitable for complexation with niflumic acid than the larger cavity of γ-CD. Bogdan *et al.*[11] using the ^{1}H NMR measurements detected the formation of 1:1 inclusion complexes between β-CD and niflumic acid caused by the insertion of trifluoromethylphenyl residue of the guest molecule into the host cavity. Ambrus *et al.*[12] proposed to use hydroxypropyl-β-CD as an additive to increase the solubility rate and *in vitro* diffusion ability of niflumic acid. The reduced gastric toxicity of niflumic acid in the presence of CD was also noted.[13] However, it should be emphasized here that quantitative data concerning interaction of α-CD with niflumic acid are absent in literature.

In this paper, we present new thermodynamic parameters of complex formation of native and hydroxypropylated α- and β-CDs with niflumic acid in aqueous solution (pH 7.4) and analyze

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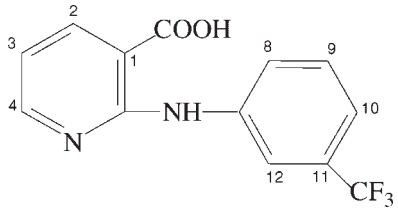

Figure 1. Structure of niflumic acid

the influence of cavity dimensions and availability of hydroxypropyl substituents on the complexation properties of CDs.

EXPERIMENTAL

Materials

Commercially available niflumic acid (Sigma), α-CD (Fluka), hydroxypropyl-α-CD (HP-α-CD, Aldrich), β-CD (Fluka), hydroxypropyl-β-CD (HP-β-CD, Aldrich) were used without further purification. CDs were stable crystallohydrates, the water content in which determined by thermogravimetric analysis was consequently taken into account during the sample preparation. Hydroxypropylated CDs with the average substitution degree 0.6 per glucose unit were randomly substituted. All measurements were performed in phosphate buffer with pH 7.4. Double distilled, deionized water was used for buffer preparation. The solutions were prepared by weight.

^{1}H NMR

All ^{1}H NMR spectra were recorded in D_2O (99.9% of isotopic purity) at pD = 7.4 and T = 298.15 K using a Bruker AC-200 spectrometer operating at 200 MHz. Cyclohexane was used as an external reference.

UV-vis spectrophotometry

Absorption spectra were recorded in the range of 200–400 nm at 298.15 K on a UV-2401 PC UV-VIS Recording Spectrometer (Shimadzu, Japan) equipped with TCC-240 A, temperature controlled cell holder. Quartz cuvettes with a path length of 1 cm were employed.

For the calculation of the binding constants, the change of absorption of niflumic acid was measured at several wavelengths as a function of CDs concentration. The concentration of niflumic acid was fixed at 5.1×10^{-5} mol kg^{-1} and the CDs concentrations were changed from 0 to 3×10^{-3} mol kg^{-1}. The CD solutions of corresponding concentration were used in the reference cuvettes.

Densimetry

The densities of solutions were measured at 298.15 K with a vibrating tube densimeter model DMA 602 (Anton Paar, Austria) thermostated to better than $\pm 1 \times 10^{-3}$ K. The densimeter was calibrated with twice-distilled water and dry air. The accuracy of measurements was $\pm 2 \times 10^{-6}$ g cm^{-3}. The niflumic acid concentration was kept constant (5×10^{-3} mol kg^{-1}), whereas the CD concentration was varied from 0 to 0.016 mol kg^{-1}.

The apparent molar volume of niflumic acid ($V_{\phi,NA}$) was calculated from the density data using the expression:

$$V_{\phi,NA} = \frac{M_{NA}}{d} - \frac{10^3 \cdot (d - d_0)}{C_{NA} \cdot d \cdot d_0} \quad (1)$$

where M_{NA} is the molecular mass of niflumic acid, C_{NA} is the concentration of niflumic acid, d and d_0 are the densities of solution and solvent, respectively. Buffer was the solvent in the binary systems (buffer + niflumic acid), whereas the solution (buffer + CD) was the solvent in the ternary system (buffer + CD + niflumic acid).

Calorimetry of solution

Measurements of the thermal effects of dissolution of crystalline samples of CD in pure buffer and in the niflumic acid solutions were performed using a home-made calorimeter of solution. The more detailed description of the construction of calorimeter was given previously.[14] All calorimetric measurements were carried out at 298.15 K. The error in the heat effect measurements was not greater than 0.6%. The concentration of niflumic acid varied from 0 to 0.017 mol kg^{-1}, and the CD concentration was fixed (1.9×10^{-3} mol kg^{-1}). Experimental enthalpies of CD dissolution in pure solvent ($\Delta H(s)$) and in the niflumic acid solution ($\Delta H(s + NA)$) were used for the calculation of enthalpies of transfer ($\Delta_{tr}H$):

$$\Delta_{tr}H = \Delta H(s + NA) - \Delta H(s) \quad (2)$$

RESULTS AND DISCUSSION

Niflumic acid is amphoteric compound with two main ionizable sites (in-ring N and COOH) and, therefore, can exist as cation, zwitterion, and anion in aqueous solutions.[15] Calculation of the equilibrium concentrations of all species using $pK_1 = 2.28$ and $pK_2 = 4.86$[15] showed that niflumic acid is fully ionized at pH > 7. Therefore, we carried out all our measurements at pH 7.4 which corresponds to (a) total ionization of niflumic acid; (b) physiological pH value; (c) higher solubility of niflumic acid.[16]

^{1}H NMR study

NMR spectroscopy can afford the information on the binding mode, stoichiometry of the complexes, and their stability. It is well known[17] that the ^{1}H NMR spectrum of α- and β-CD in D_2O consists of the signals of H(1), H(2), H(3), H(4), H(5), and H(6) protons. It is necessary to note that protons H(1), H(2), H(4), and H(6) are located on the exterior of CD cavity, whereas protons H(3) and H(5) are placed inside macrocyclic cavity near the wider and narrow rims of CD molecule, respectively. Protons H(3) and H(5) are sensitive to the inclusion complex formation. Addition of the excess amount of niflumic acid (0.016 mol kg^{-1}) to the solutions of α-CD (0.005 mol kg^{-1}) and β-CD (0.005 mol kg^{-1}) induces the measurable shifts of the resonances of H(3) and H(5) protons confirming the inclusion complex formation process. The chemical shift changes are larger for β-CD protons due to the formation of more stable complex in this system. Figure 2, as an example, shows the ^{1}H NMR spectra of β-CD in the free state and in the presence of niflumic acid. It should be noted that the chemical shift changes of the external protons of both α-CD and β-CD are insignificant. Thus, the results of the qualitative ^{1}H NMR

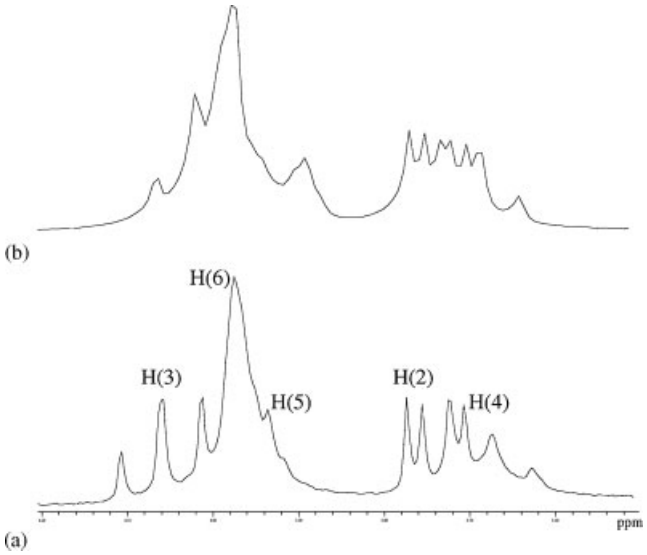

Figure 2. The partial ^{1}H NMR spectrum of β-CD alone (a) and in the presence of niflumic acid (b) at 298.15 K

analysis indicate the penetration of niflumic acid inside the CD cavity.

^{1}H NMR spectra of niflumic acid (0.005 mol kg^{-1}) in the presence of CDs were also considered. Figure 3 illustrates the changes of the chemical shifts of niflumic acid protons with respect to increasing CD concentration. The shape of the titration curves in Fig. 3 points out the complex formation of niflumic acid with all CDs under study. The 1:1 stoichiometry of the β-CD/niflumic acid and HP-β-CD/niflumic acid complexes was determined by Bogdan *et al.*[11] and by us in our recent publication,[9] respectively. Moreover, according to the Benezi–Hildebrand method,[18,19] dependences presented in Fig. 3 were converted into double reciprocal plots (Fig. 4), the linearity of which additionally confirms the 1:1 binding mode.

The 1:1 complexation of niflumic acid with CDs can be described as follows:

$$CD + NA = CD \cdot NA \tag{3}$$

Equilibrium constant is written as

$$K = \frac{[CD \cdot NA]}{[CD] \cdot [NA]} = \frac{[CD \cdot NA]}{(C_{CD} - [CD \cdot NA]) \cdot (C_{NA} - [CD \cdot NA])} \tag{4}$$

where C_{CD} and C_{NA} are the initial concentrations of CD and niflumic acid, respectively.

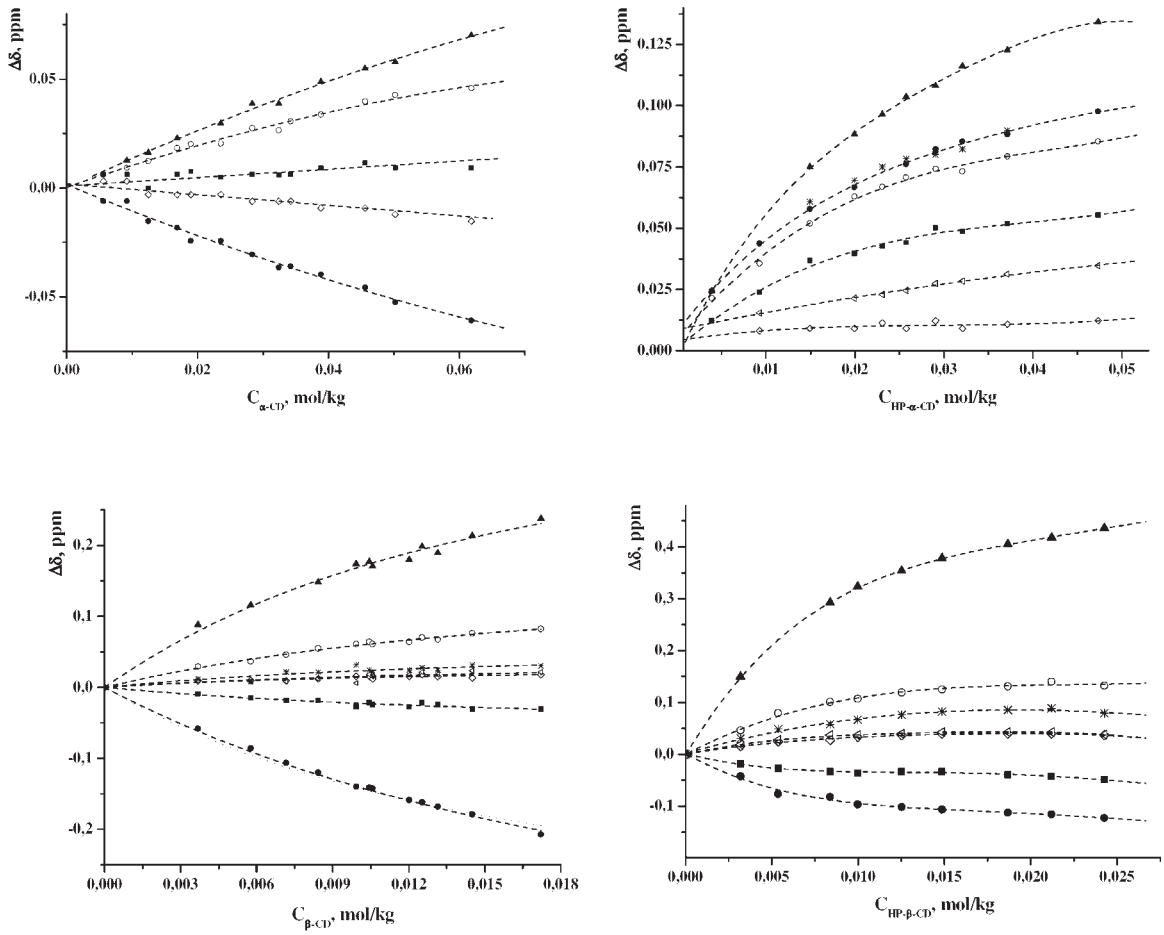

Figure 3. Dependences of the chemical shift changes of niflumic acid protons *versus* the cyclodextrin concentration (* - H(2), M - H(3), Ξ - H(4), ! - H(8), – - H(9), 7 - H(10), , - H(12))

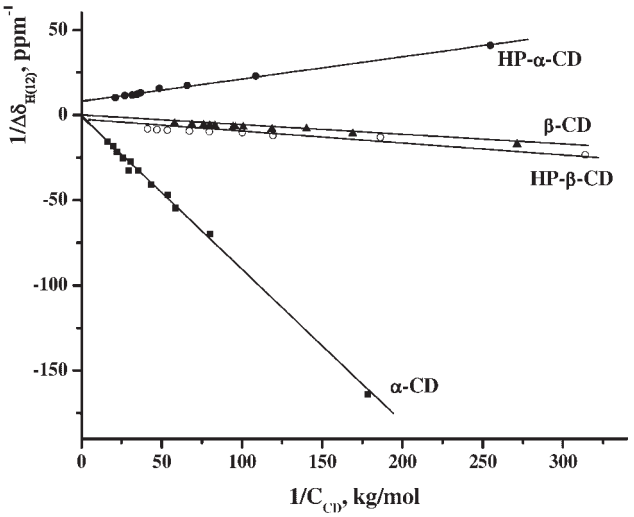

Figure 4. Double reciprocal plots for complex formation of niflumic acid with cyclodextrins

The observed chemical shift (δ_{exp}) for niflumic acid protons can be expressed as follows

$$\delta_{exp} = \alpha \cdot \delta_f + (1 - \alpha) \cdot \delta_c \quad (5)$$

where α is the fraction of niflumic acid in the free state; δ_f and δ_c are the chemical shifts for free and complexed niflumic acid, respectively.

Letting $\Delta\delta = \delta_{exp} - \delta_f$ and $\Delta_c\delta = \delta_c - \delta_f$, and after some re-arrangements of Eqns (4) and (5) one can obtain

$$K = \frac{\Delta\delta \cdot C_{NA}}{\Delta_c\delta \cdot (C_{CD} - C_{NA} \cdot \Delta\delta/\Delta_c\delta) \cdot (C_{NA} - C_{NA} \cdot \Delta\delta/\Delta_c\delta)} \quad (6)$$

The values of K and $\Delta_c\delta$ determined from nonlinear fitting of the data using Eqn (6) are listed in Tables 1 and 2, respectively.

As follows from Table 1, α-CDs possessing the smaller cavity diameter form less stable complexes with niflumic acid. The presence of hydroxypropyl substituents in the CD molecule promotes the binding and makes the inclusion complexes more stable. In particular, this is well noticeable on the complexes with HP-α-CD. Perhaps, the stronger binding affinity of modified CDs to niflumic acid is originated by their higher flexibility permitting to hold the guest molecule.

As can be seen from Table 2, the signals of protons of trifluoromethylphenyl residue are significantly shifted upon complex formation with all considered CDs. This fact illustrates that trifluoromethylphenyl residue of niflumic acid molecule penetrates into CD cavity. The measurable $\Delta_c\delta$ values obtained for H(2), H(3), and H(4) protons (Table 2) confirm that insertion of the pyridine ring into macrocyclic cavity can also take place. Thus, relying on the obtained results, we can assume the co-existence of two kinds of 1:1 complexes. In one of them, the trifluoromethylphenyl residue is located in the CD cavity, and in the second one—the pyridine ring is placed inside the cavity.

Comparison of $\Delta_c\delta$ values obtained for complexes with α-CD and β-CD (Table 2) shows that the cavity size has no influence on the binding mode. Nevertheless, the magnitudes of $\Delta_c\delta$ are lower for less stable complexes formed by α-CD. Considering the complex formation of niflumic acid with native and substituted CDs, we can note that the $\Delta_c\delta$ values for protons of pyridine ring are higher in the case of binding with the modified CDs (Table 2). It means that the fraction of complexes in which the pyridine ring is included into macrocylic cavity is slightly increased upon complex formation of niflumic acid with hydroxypropylated α- and β-CD.

UV-vis spectroscopic study

The absorption spectra of niflumic acid in the absence and in the presence of β-CD and HP-β-CD are shown in Fig. 5. Two absorption maximum wavelengths were observed at 204 and 286.5 nm. By adding CDs, there was a small bathochromic shift for the second absorption maximum, reaching 287 and 288.5 nm at the maximum concentration of β-CD and HP-β-CD, respectively. The presence of β-CD and HP-β-CD results in decrease and increase in the absorbance intensity, respectively. The spectral changes induced by the addition of CDs indicate the transfer of niflumic acid from the aqueous environment to the apolar CD cavity upon inclusion complex formation. The discrepancy observed for the influence of native and modified β-CD on the absorbance intensity can be attributed to the formation of different types of 1:1 inclusion complexes. This assumption is proved by the above discussed ^{1}H NMR data, according to which the fraction of the complexes with included pyridine ring is increased upon complex formation with HP-β-CD.

In order to evaluate the stability constants of the complexes we measured the change in the absorbance (ΔA) with increasing concentrations of CDs at several wavelengths. As an example, the concentration dependences of ΔA are presented in Fig. 6. The stability constants were calculated from nonlinear fitting of the

Table 1. Apparent stability constants of the complexes of niflumic acid with cyclodextrins (phosphate buffer with pH = 7.4, T = 298.15 K)

	lg*K*				
CD	Calorimetry	Densimetry	UV	^{1}H NMR	Literature
α-CD	0.13 ± 0.09*	—	—	0.9 ± 0.2	
HP-α-CD	—	—	—	1.8 ± 0.2	
β-CD	2.5 ± 0.1	2.4 ± 0.4	2.6 ± 0.1	2.0 ± 0.3	2.72 (pH 7)[10] 2.53 (pH 12)[11]
HP-β-CD	2.2 ± 0.1	2.8 ± 0.4	3.09 ± 0.02	2.3 ± 0.1	

* Standard deviation calculated by the fitting program (the same is for Tables 2 and 3).

Table 2. Chemical shift changes of niflumic acid protons induced by 100% complex formation with cyclodextrins (phosphate buffer with pH = 7.4, $T = 298.15$ K)

	$\Delta_c\delta$, ppm						
CD	H(2)	H(3)	H(4)	H(8)	H(9)	H(10)	H(12)
α-CD	$\Delta\delta < 0.01$	-0.04 ± 0.01	$\Delta\delta < 0.01$	0.04 ± 0.01	0.15 ± 0.04	0.21 ± 0.01	-0.19 ± 0.01
HP-α-CD	0.13 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.12 ± 0.01	0.19 ± 0.03	0.13 ± 0.01
β-CD	0.06 ± 0.03	0.03 ± 0.01	0.04 ± 0.01	-0.06 ± 0.02	0.16 ± 0.01	0.41 ± 0.04	-0.34 ± 0.01
HP-β-CD	0.10 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	-0.05 ± 0.01	0.16 ± 0.01	0.53 ± 0.01	-0.14 ± 0.01

data to Benesi–Hildebrand equation:[18]

$$A = A_0 + \frac{\Delta\varepsilon \cdot K \cdot C_{NA} \cdot C_{CD}}{1 + K \cdot C_{CD}} \quad (7)$$

where A and A_0 are the absorbance of niflumic acid in the presence and absence of CD, respectively; $\Delta\varepsilon = \varepsilon_{CD \cdot NA} - \varepsilon_{NA}$ is the difference in the molar absorptivities between free (ε_{NA}) and complexed ($\varepsilon_{CD \cdot NA}$) niflumic acid; C_{NA} and C_{CD} are the concentrations of niflumic acid and CD, respectively; K is the stability constant of the complex. Additionally, K values were calculated with the aid of nonlinear least-squares analysis implemented in computer program. Results of calculation are listed in Table 1.

As it is evident from Table 1, the K values are slightly higher than those obtained by the other methods. The similar result was observed by Canipelle *et al.*[20] for complex formation of β-CD with monosulfonated triphenylphosphine oxides in the aqueous solution. The different K values were also obtained by Caron *et al.*[21] for complex formation between the sodium salt of the monosulfonated triphenylphosphine and the β-CD investigated in the aqueous solution by high field nuclear magnetic resonance and UV-vis spectroscopies.

The variance of K can be explained by small spectral changes, which increase the influence of experimental errors.[22] The second kind of errors can be caused by the optical presence of CD, which gives the irreproducible values of absorbance.[22] Finally, the errors can arise from the incorrect treatment of the experimental data. For example, Paduano *et al.*[23] received different K values from ^{1}H NMR and UV measurements owing to the use of wrong binding model for calculation. To exclude the last source of errors we consider the various binding models. Since niflumic acid contain two aromatic rings in the structure, the possibility of participation of both rings in complex formation with two CD molecules should be examined. Therefore, the spectroscopic data were additionally fitted for 2:1 (host–guest)

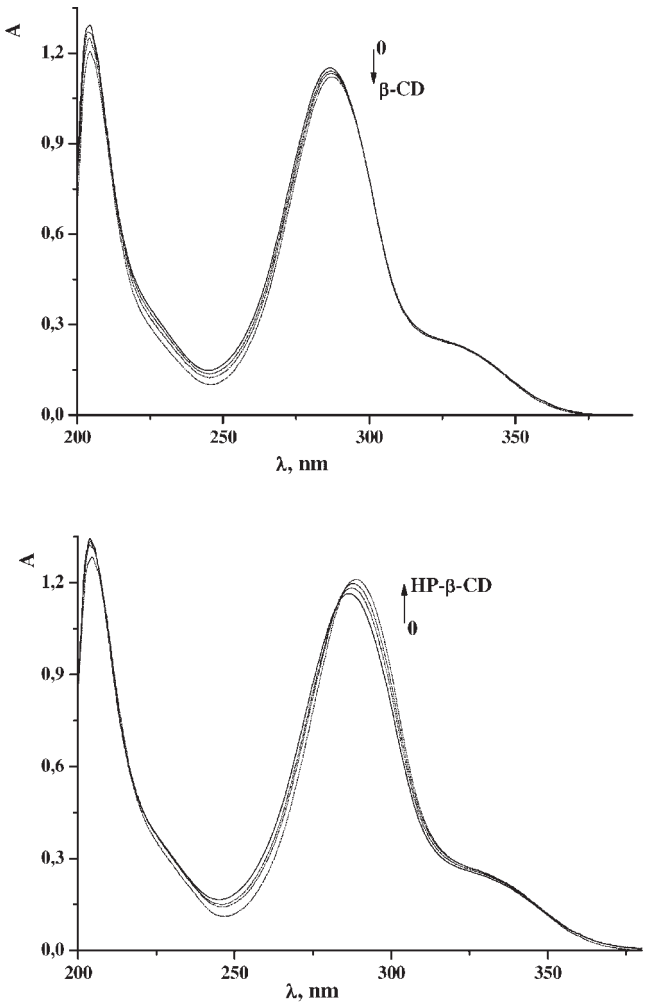

Figure 5. Absorption spectra of niflumic acid (5×10^{-5} mol kg^{-1}) in the absence and presence of the excess amounts of β-CD and HP-β-CD (pH = 7.4; $T = 298.15$ K)

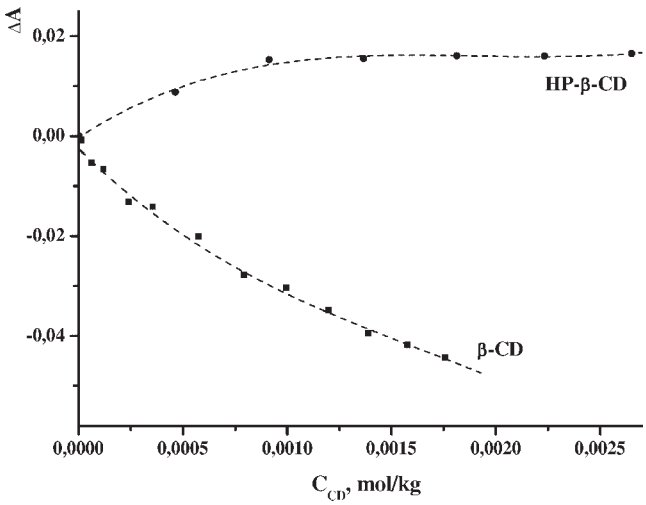

Figure 6. Changes in the absorption of niflumic acid *versus* the cyclodextrin concentration (pH = 7.4; $T = 298.15$ K λ = 285nm)

binding model according to the following equation:

$$A = A_0 + \frac{K_{1:1} \cdot \varepsilon_{CD \cdot NA} \cdot C_{NA} \cdot C_{CD} + K_{2:1} \cdot \varepsilon_{(CD)_2 \cdot NA} \cdot C_{NA} \cdot C_{CD}^2}{1 + K_{1:1} \cdot C_{CD} + K_{1:1} \cdot K_{2:1} \cdot C_{CD}^2} \quad (8)$$

where $\varepsilon_{CD \cdot NA}$ and $\varepsilon_{CD_2 \cdot NA}$ are the molar absorptivities of the 1:1 and 2:1 complexes, respectively; $K_{1:1}$ and $K_{2:1}$ are the stability constants of 1:1 and 2:1 complexes, respectively. The $K_{2:1}$ values obtained by nonlinear fitting of the data using Eqn (8) were incorrect from the thermodynamic point of view ($K_{2:1} < 0$). Thus, best fitting was obtained for 1:1 complexation model. In addition to the Job plots,[9,11] double reciprocal plots (Fig. 4), this result proves the formation of 1:1 complexes between niflumic acid and CDs.

Volumetric study

The transfer of niflumic acid from the aqueous solution to the apolar cavity of CD should be reflected in its molar volume. For this purpose, the apparent molar volumes of niflumic acid were determined at different concentrations of β-CD and HP-β-CD. The obtained concentration dependences of $V_{\phi,NA}$ are presented in Fig. 7. It can be seen from Fig. 7 that significant nonlinear increase in $V_{\phi,NA}$ values with increase of CD concentration is observed for both systems. According to Young's rule,[24] the apparent molar volume contains the contributions from free and complexed species. Concerning the 1:1 complex formation, the experimental $V_{\phi,NA}$ value can be defined as

$$V_{\phi,NA} = \alpha \cdot V_{\phi,NA_f} + (1 - \alpha) \cdot V_{\phi,NA_c} \quad (9)$$

where α is the fraction of free niflumic acid; V_{ϕ,NA_f} and V_{ϕ,NA_c} are the apparent molar volumes of free and fully complexed niflumic acid, respectively. After some re-arrangements Eqn (9) can be transformed into Eqn (10):

$$V_{\phi,NA} = \frac{V_{\phi,NA_f} + K \cdot V_{\phi,NA_c} \cdot C_{CD}}{1 + K \cdot C_{CD}} \quad (10)$$

where K is the stability constant and C_{CD} is the CD concentration. By applying Eqn (10) to the data in Fig. 7 using a nonlinear least-squares fitting method, the V_{ϕ,NA_c} and K values were obtained. It is necessary to note here that K values determined from the density measurements are in agreement with K obtained by the other experimental methods (Table 1). The tendency of K to be higher for complexes with substituted CD is also observed.

Calculated V_{ϕ,NA_c} values corresponding to 100% complex formation of niflumic acid with β-CD and HP-β-CD are equal to 229 ± 2 and 226 ± 1 cm^3 mol^{-1}, respectively. These values are considerably higher than $V_{\phi,NA_f} = 194.4$ cm^3 mol^{-1} corresponding to the niflumic acid in the free state. The difference between V_{ϕ,NA_c} and V_{ϕ,NA_f} defined as the volume of transfer ($\Delta_{tr}V$) is positive. In spite of the limited number of publication devoted to volumetric study of inclusion complexes of CDs, the positive volumes of transfer have also been obtained for the complex formation of β-CD with α, ω-alkyl dicarboxylate anions,[25] gallic acid,[26] alkane-α, ω-diols,[27] and 2-naphthyl acetate.[28]

Franks *et al.*[29] and Shahidi *et al.*[30] pointed out that the partial molar volume of nonelectrolyte ($\overline{V}^0$) is determined by the intrinsic volume of solute and the volume changes due to its interactions with the solvent:

$$\overline{V}^0 = V_{vw} + V_{void} - V_{shrinkage} \quad (11)$$

where V_{vw} is van der Waals volume, V_{void} is the associated void or empty volume, $V_{shrinkage}$ is the shrinkage in volume caused by the interaction of hydrogen bonding groups of the solute with the water molecules. Assuming that V_{vw} and V_{void} of the niflumic acid are the same in water and CD solution, the positive volume of transfer can be explained by the decrease in volume of shrinkage in the presence of CD in the aqueous solution. β-CD is known as structure-breaker solute.[31] Thus, the observed results indicate that the structure-breaking effect of β-CDs decreases upon binding with niflumic acid. It means that more water molecules are released in the bulk water, which has higher volume contribution than structure-broken water.[32] Moreover, the partial or complete expulsion of solvent molecules located inside the CD cavity to the bulk solvent results in the positive contribution to $\Delta_{tr}V$.[25,27]

The V_{ϕ,NA_c} values obtained for the complex formation of niflumic acid with β-CD and HP-β-CD are practically equal, within the error margins. This fact allows to suppose that the number of solvent molecules released from the CD cavity to the bulk solvent and contribution from this process should be similar for β-CD and HP-β-CD. As a consequence, the volume changes caused by the reorganization of solvent molecules upon complex formation are approximately the same for both CDs.

Calorimetric study

Calorimetry is a direct method to obtain all thermodynamic parameters of complex formation (K, $\Delta_c G$, $\Delta_c H$, and $\Delta_c S$) and to

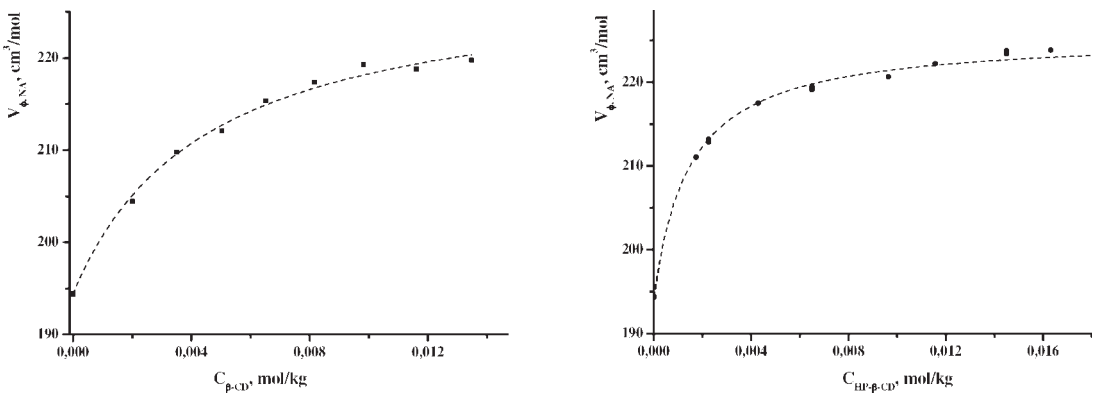

Figure 7. Apparent molar volumes of niflumic acid *versus* the cyclodextrin concentration (pH = 7.4; T = 298.15 K)

Journal of Physical Organic Chemistry

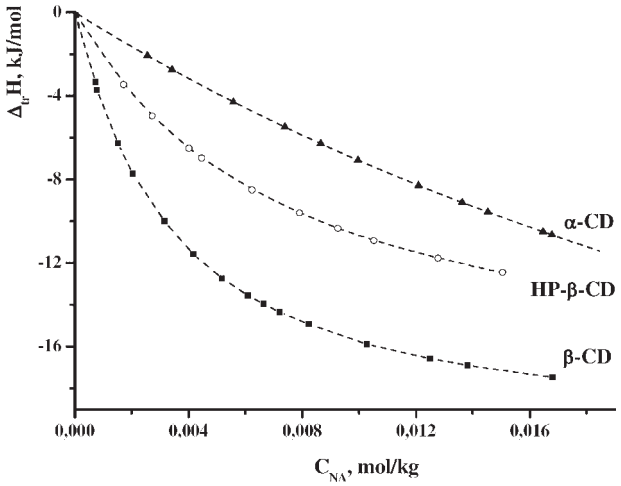

Figure 8. Isotherms of binding of cyclodextrins with niflumic acid (pH = 7.4; T = 298.15 K)

predict the driving forces of binding. Isotherms of binding are shown in Fig. 8. Mathematical treatment of these dependences using minimization computer program HEAT[33] allows to simultaneously estimate the stability constant (Table 1) and the enthalpy of complex formation (Table 3). Free energy and entropy of complex formation reported in Table 3 were calculated from the well-known thermodynamic equations:

$$\Delta_c G = -\mathrm{RT} \ln K \tag{12}$$

$$\Delta_c G = \Delta_c H - T\Delta_c S \tag{13}$$

The process of complex formation of CDs involves the following stages: destruction of solvation shells of the solutes; release of cavity-bound water, host–guest interactions (hydrogen bonding, hydrophobic, electrostatic, van der Waals interactions), and hydration of the complex.[4,34] Thermodynamic parameters of complex formation reflect the contributions from all these processes.

It was obtained that inclusion of niflumic acid into CD cavity is accompanied by the negative enthalpy and entropy changes (Table 3), so it is enthalpy driven. The high negative values of enthalpy can result from several contributions, which predominate over the hydrophobic interactions and desolvation. First of them is exothermic effect from van der Waals interactions and possible hydrogen bonding. The second one is the exothermic effect from the exclusion of solvent molecules from the CD cavity.[34] The cavity-bound water ("enthalpy-rich" or "activated" water) is at a higher energy than is bulk water. Therefore, its release upon the CD complexation is enthalpy favorable.[34]

Complex formation of niflumic acid with α-CD is characterized by lower K and more negative $\Delta_c H$ and $\Delta_c S$ values (Table 3). Due to very small stability constant and practically linear binding isotherm, it was problematic to calculate the thermodynamic parameters of complex formation more precisely for this system. Low stability of this complex is a result of enthalpy–entropy compensation. As follows from ^{1}H NMR data, the insertion of niflumic acid in the α-CD cavity takes place. More probably, the large negative enthalpy of complexation indicates the prevalence of van der Waals interactions instead of the hydrophobic interactions. Smaller α-CD cavity is more suitable for tight placement of niflumic acid molecule. In this case, aromatic rings of niflumic acid are in close van der Waals contact with the cavity walls, and this phenomenon results in favorable enthalpy contribution. In addition, the polar groups of niflumic acid remained outside the cavity can form H-bonds with OH-groups surrounding the rims of α-CD molecule. Large negative entropy value obtained for this system confirms the formation of more compact and less flexible structure.

With respect to α-CD, the thermodynamic parameters obtained for complex formation with β-CDs are considerably higher (Table 3). The decrease in the exothermicity of binding caused by the increased positive contribution from the dehydration effects is confirmed by less negative entropy changes. The proximity of thermodynamic parameters obtained for niflumic acid complexation with β-CD and HP-β-CD can point out the similar binding mode in these systems. This conclusion is in accordance with the results of densimetry and ^{1}H NMR.

Table 3. Apparent thermodynamic parameters of complex formation of niflumic acid with cyclodextrins (phosphate buffer with pH = 7.4, T = 298.15 K)

CD	$\Delta_c G$, kJ mol^{-1}	$\Delta_c H$, kJ mol^{-1}	$\Delta_c S$, J (mol K)$^{-1}$
α-CD	-0.7 ± 0.5	-40 ± 1	-132 ± 97
β-CD	-14.5 ± 0.6	-22.1 ± 0.5	-25 ± 2
HP-β-CD	-12.3 ± 0.6	-20.1 ± 0.6	-26 ± 2

CONCLUSIONS

The results of this work demonstrated that native and hydroxypropylated α- and β-CDs are able to form with niflumic acid the 1:1 inclusion complexes. Insertion of both phenyl and pyridine residues of niflumic acid molecule in the macrocyclic cavity of the all considered CDs takes place. However, the inclusion of the former residue is more preferential. The β-CD was found to be a more suitable complexating agent for niflumic acid since it forms more stable inclusion complexes with it.

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