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Experimental Analysis of Complex Formation of Niflumic Acid with β-Cyclodextrins

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Abstract

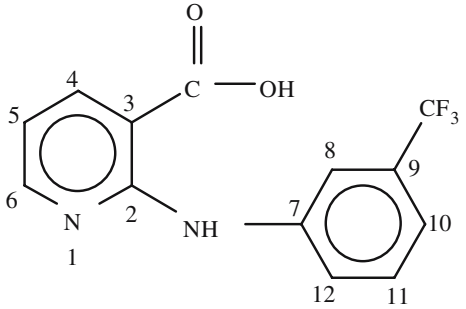
Complex formation of niflumic acid with β-, hydroxypropyl-β- and methyl-β-cyclodextrins in aqueous solution (pH 7.4) were studied by calorimetry of solution, ^{1}H NMR spectroscopy and solubility method. The enhancement of niflumic acid solubility in the presence of hydroxypropyl-β-cyclodextrin was detected. This effect is explained on the basis of ^{1}H NMR data confirming the inclusion of hydrophobic trifluoromethylphenyl residue of niflumic acid molecule into the macrocyclic cavity. The thermodynamic parameters of 1:1 binding were derived from the data of calorimetry and solubility measurements. It was obtained, that complex formation of niflumic acid with β-cyclodextrin and both its derivatives is enthalpy driven. Substitutes surrounding the macrocyclic cavity slightly influence the thermodynamics of complex formation resulting in decrease of stability of the complexes formed.

Introduction

Cyclodextrins (CDs) are the macrocyclic receptors, which are widely adopted in supramolecular chemistry due to their unique structure and properties [1]. It is well known, that CD is a toroidal shape molecule with hydrophilic outer and partially hydrophobic cavity that is capable to include different guest molecules [1–3]. Cyclodextrins complexes have received the numerous practical applications in cosmetic, textile, food, pharmaceutical industries, as well as in the separation technologies [4]. In particular, complex formation of drugs with CDs gives opportunity to solve some delicate problems of medical and pharmaceutical chemistry concerning the development and design of effective medicines [4]. Moreover, CDs can be used to improve physicochemical properties of drugs (solubility, bioavailability, stability, etc.) and to reduce their unwanted side effects.

Our attention was focused on the complex formation of CDs with niflumic acid (2-[[3-(trifluoromethyl)-phenyl]-amino]-3-pyridinecarboxylic acid), which is a representative of the class of non-steroidal anti-inflammatory drugs. In spite of numerous positive effects of niflumic acid (NA), there are a number of disadvantages. First, it is very low solubility of the NA in aqueous solutions that restricts their using in parenteral formulations [5, 6]. Second, NA displays undesirable side effects such as gastric irritation, asthenia, bleeding and etc. [7]. Complex formation of NA with CDs can be employed as one from the ways to reduce its toxicity and to increase its solubility and anti-inflammatory activity [8].

Analysis of the available literature data shown, that α-CD has no affinity to complex formation with NA in aqueous solution, whereas β-CD exhibits opposite behavior [9]. Bogdan *et al.* [10] confirmed the process of 1:1 inclusion complex formation between β-CD and niflumat anion by ^{1}H NMR and X-ray methods. The obtained results testify a preference for insertion of the trifluoromethylphenyl residue, rather than the pyridinecarboxylate moiety in the hydrophobic cavity from the secondary and primary sides of β-CD molecule. Indicated mode of binding is observed as in solution (alkaline medium, pH 12) as well as in the crystalline



Scheme 1. Scheme of niflumic acid.

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complex. However, complex formation of NA with modified CDs was not studied.

We report a new data on complex formation of NA with modified β-CDs such as hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin in aqueous solution obtained by calorimetry of solution, ^{1}H NMR and solubility method. The application of derivatized CDs is governed by their higher solubility in water compared to unsubstituted CDs. Substituents can also change the cavity dimensions, as well as the hydrophobicity, polarity and CD ability to H-bonding. So, the selectivity of complex formation and stability of the complexes of modified CDs can differ from those of native CDs. Therefore, the aims of our study were to compare the complexation ability of native and modified β-CDs towards the NA and to analyze the influence of availability and the nature of substituents in CD molecule on the thermodynamics of complex formation and the binding mode.

Experimental

Materials

Niflumic acid ((2-[[3-(trifluoromethyl)-phenyl]-amino]-3-pyridinecarboxylic acid), NA, $C_{13}H_9F_3N_2O_2$, FW 282.22, Sigma Lot 12K1486, purity min 98.0%) was used as received. 2-Hydroxypropyl-β-CD (HP-β-CD), with molar substitution 0.58–0.73 per glucose unit was a generous gift from Wacker–Cheme GmbH, München, Germany. Methyl-β-cyclodextrin (M-β-CD) was a mixture of randomly substituted components (Aldrich) with molar substitution 1.6–2.0 per glucose and it was used as received. CDs were stable crystallohydrates, the water content in which determined by thermogravimetry (heating of samples from 298 to 433 K) was 14, 8 and 3% for β-CD, HP-β-CD and M-β-CD, respectively and was considered when calculating CDs concentrations. All chemicals used in preparation of the buffers and solutions were analytical reagent grade.

Water was doubly distilled prior to use. All solutions were prepared by weigh. The measurements were performed at pH 7.4 (phosphate buffer), which mimics the physiological conditions and corresponds to better solubility of the NA in aqueous solution [6].

Methods

Solubility method

Solubility measurements were performed in phosphate buffer (pH 7.4) at different temperature 298, 303, 310 and 315 K. An excess amount of NA was added to buffer solution containing various concentrations of HP-β-CD (0–0.015 mol kg^{-1}). The prepared solutions were stirred at each temperature for 48 h until equilibrium was achieved. After equilibration the solutions were centrifuged and the solid phase was removed by filtration (Acrodisc CR syringe filter, PTFE, 0.2 μm pore size) in a special temperature-controlled box. The concentration of NA was determined spectrophotometrically (Specord CΦ–46) at 288 nm ($\varepsilon = 29302$ kg·(mol cm)$^{-1}$). Each experiment with given CD concentration was repeated at least three times.

^{1}H NMR

A Bruker AC-200 spectrometer (200 MHz) was used to record the proton NMR spectra of NA samples in pure D_2O and then in the HP-β-CD + D_2O solutions at pH 7.4 and $T = 298.15 \pm 0.10$ K. Cyclohexane was employed as external reference. NA signals in ^{1}H MNR spectra (δ_{NA}) were assigned on the basis of available in the literature data [10].

Calorimetry of solution

Solid CDs samples of constant mass (concentration is 8×10^{-4} mol kg^{-1}) were dissolved at first in pure buffer solution and then in buffer + NA solutions of variable concentration of NA (from 0.001 to 0.016 mol kg^{-1}). The thermal effects of solution were detected by means of home made isothermal calorimeter at 298.15 K. The calorimetric installation consists of a calorimetric cell volume of 17 ml containing a temperature gage (thermistor), a calibrated heater, a titanium stirrer and ampoule holder; a mercury seal to prevent heat loss; systems of air and liquid thermostatting. A thermistor was connected with a resistance bridge and a recorder potentiometer. An electrical calibration was carried out before and after each experiment. The error in the heat effect measurements was not greater than 0.6%.

Results

The solubility of NA was determined at various HP-β-CD concentrations and different temperatures. Figure 1 shows the phase solubility diagram of NA in HP-β-CD solutions at different temperatures.

As it is follows from Figure 1, NA solubility is increased proportionally to the enhancement of HP-β-CD concentration. The NA solubility in HP-β-CD solution of concentration 0.0145 mol kg^{-1} is higher than those for the pure buffer solution (pH 7.4) by a factor about 2.5 at the temperatures studied.

Considerable enhancement of solubility was archived due to complex formation of NA with HP-β-CD. According to the Higuchi and Connors classification [11], the linear solubility diagrams of A_L type (Figure 1) indicate formation of a soluble 1:1 complex.

The 1:1 composition of complex was also confirmed by ^{1}H NMR spectroscopy. The chemical shift changes of the NA protons at the complex formation are listed in Table 1. As can be seen from Table 1, the signals of the NAs protons (with the exception of H(8) and H(12)) are shifted downfield in the presence of equimolar amount of HP-β-CD. The shifting is more noticeable for H(10)

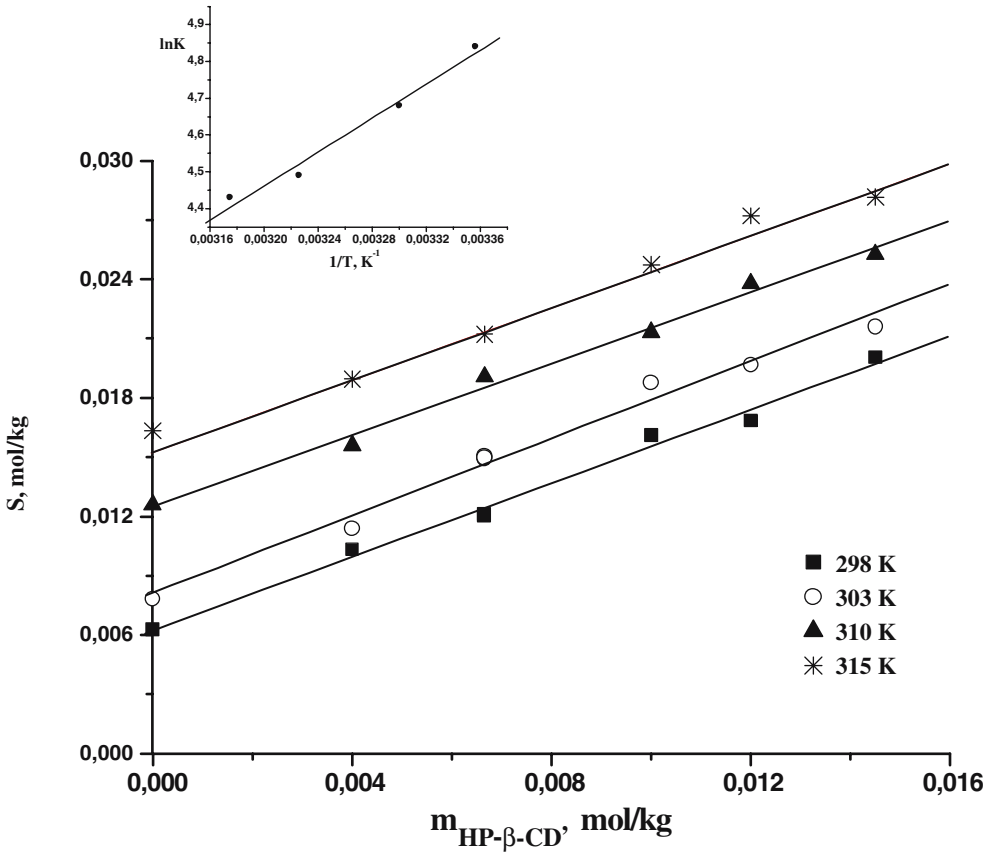

Figure 1. Solubility phase diagrams of NA in HP-β-CD solution at different temperatures. Inset: Van't Hoff plot for calculation of the enthalpy of complex formation between NA and HP-β-CD.

and H(11) protons. Therefore, chemical shifts of H(10) and H(11) NA protons were used for obtaining Job-plots. Plotting $\Delta\delta_{NA} \cdot R$ against R (where $R = [NA]/([NA] + [HP\text{-}\beta\text{-}CD])$ [12] results in the curve with maxima at $R = 0.5$ (Figure 2) indicating 1:1 composition of the inclusion complex. Symmetrical shape of the curve presented in Figure 2 suggests the presence of complexes with the single 1:1 stoichiometry.

Binding constants were obtained on the basis of solubility diagrams. In this case the stability constant can be calculated from the following equation [11]:

$$S = S_0 + K \cdot S_0 \cdot [CD] \qquad (1)$$

where S and S_0 are solubility of NA in buffer solution with and without CD, respectively; K is binding constant; [CD] is CD concentration.

Other thermodynamic parameters ($\Delta_c G$, $\Delta_c H$, $\Delta_c S$) of the complex formation determined from the temperature dependence of the association constant K using the van't Hoff relation (Figure 1, inserted graph) are listed in Table 2.

Table 2 also includes thermodynamic characteristics of complex formation of NA with β-CD, HP-β-CD and M-β-CD obtained by direct calorimetric method. In this

Table 1. ^{1}H MNR chemical shift changes ($\Delta\delta = \delta_{complexed} - \delta_{free}$) of NA protons upon complex formation with HP-β-CD ($T = 298$ K; $c_{NA} = 0.0055$ mol · kg^{-1}; $c_{HP\text{-}\beta\text{-}CD} = 0.0055$ mol · kg^{-1})

Proton	$\Delta\delta$/ppm
H(4)	0.07
H(5)	0.03
H(6)	0.03
H(8)	−0.05
H(10)	0.24
H(11)	0.09
H(12)	−0.01

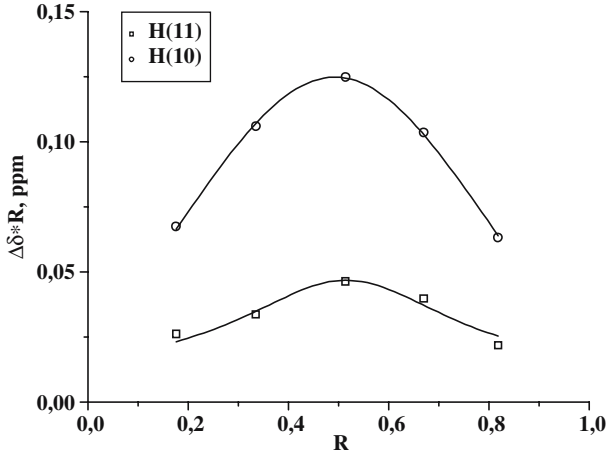

Figure 2. Job plot for NA/HP-β-CD complex.

Table 2. Thermodynamic parameters of NA complex formation with β-CDs in combination with the literature data ($T = 298.15$ K; [a]Units: kJ mol^{-1})

Complex	Method	lg$K$	$\Delta_c G^a$	$\Delta_c H^a$	$T\Delta_c S^a$
β-CD/NA	Calorimetry (pH 7.4)	2.5 ± 0.1	-14.5 ± 0.6	-22.1 ± 0.5	-7.6 ± 0.5
β-CD/NA [9]	Calorimetry (pH 7.0)	2.72	−15.5	−19.0	−3.5
β-CD/NA [9]	Chromatography (pH 6.0)	3.05	–	–	–
β-CD/NA [10]	^{1}H NMR (pH 12)	2.44	–	–	–
HP-β-CD/NA	Calorimetry (pH 7.4)	2.2 ± 0.1	-12.3 ± 0.6	-20.1 ± 0.6	-7.8 ± 0.6
HP-β-CD/NA	Solubility (pH 7.4)	2.1 ± 0.1	-12.0 ± 0.6	-19 ± 2	-7 ± 1
M-β-CD/NA	Calorimetry (pH 7.4)	2.0 ± 0.5	-11 ± 3	-28 ± 1	-17 ± 5

case experimentally determined enthalpies of solution were used for calculation of enthalpies of transfer of CDs from pure solvent to the NA solution ($\Delta_{tr}H(s \rightarrow s+y)$) according to the following equation:

$$\Delta_{tr}H(s \rightarrow s+y) = \Delta_s H(s+y) - \Delta_s H(s) \qquad (2)$$

where $\Delta_s H(s+y)$ is the enthalpy of solution of CD in NA solution ($s+y$) and $\Delta_s H(s)$ is the enthalpy of solution of CD in pure solvent (s).

Equilibrium constants (K) and enthalpies of complex formation ($\Delta_c H$) were calculated on the basis of initial concentrations of reagents and experimental values of enthalpies of transfer by means of computer program HEAT in which a search of unknown parameters is reduced to the numerical minimization of the F functional [13]:

$$F = \sum_{i=1}^{N} W_i \cdot (\Delta H_{i,\mathrm{exp}} - \Delta H_{i,\mathrm{calc}})^2 \qquad (3)$$

where ΔH_i is the thermal effect of the i-reaction, N is the number of experiments, w_i is the weighted factor. Examples of the fit between values calculated in this way and the experimental values are shown in Figure 3.

Calculated stability constants and all other thermodynamic parameters are the apparent quantities since we used concentrations instead of activities and ionic strength was not equal to zero.

Discussion

The NA solubility increased linearly as the HP-β-CD concentration increased. The enhancement in solubility can be attributed to the formation of inclusion complex between NA and HP-β-CD. This complex has a higher solubility due to hydrophilic exterior of CD molecule and insertion of hydrophobic part of NA molecule into macrocyclic cavity. Information on the binding mode of NA with HP-β-CD was obtained from a ^{1}H NMR study. As it follows from Table 1, the NA protons that are located in the benzene ring are more shifted. These results confirm the inclusion of trifluoromethylphenyl moiety inside the HP-β-CD cavity.

The linear phase solubility diagrams of NA in aqueous HP-β-CD solutions with a slope less than 1 (Figure 1) and results of ^{1}H NMR analysis (Figure 2) testify the formation of complex of 1:1 stoichiometry. Therefore, the process of complex formation between NA and CD is given by the following equilibrium:

$$\mathrm{CD} + \mathrm{NA} \overset{\mathrm{K}}{=} \mathrm{CD} \times \mathrm{NA} \qquad (4)$$

where K is equilibrium constant (or stability constant) which can be written as

$$K = [\mathrm{CD} \times \mathrm{NA}]/([\mathrm{CD}] \cdot [\mathrm{NA}]) \qquad (5)$$

The calculated and available in literature thermodynamic parameters of complex formation of NA with CDs are presented in Table 2. As follows from Table 2, the complexes of NA with CDs under study are stable and they are characterized by the favorable enthalpy changes ($\Delta_c H < 0$) and unfavorable entropy changes ($\Delta_c S < 0$). So, complexes are stabilized by the enthalpy term of Gibbs energy.

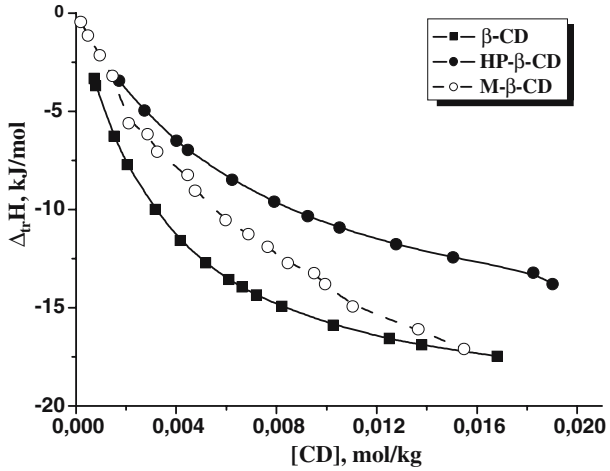

Figure 3. Calorimetric binding isotherms for the interactions of NA with β-CD and its derivatives in water at $T = 298.15$ K and pH = 7.4 (symbols denote the experimentally obtained points; the curves are calculated for 1:1 complex formation).

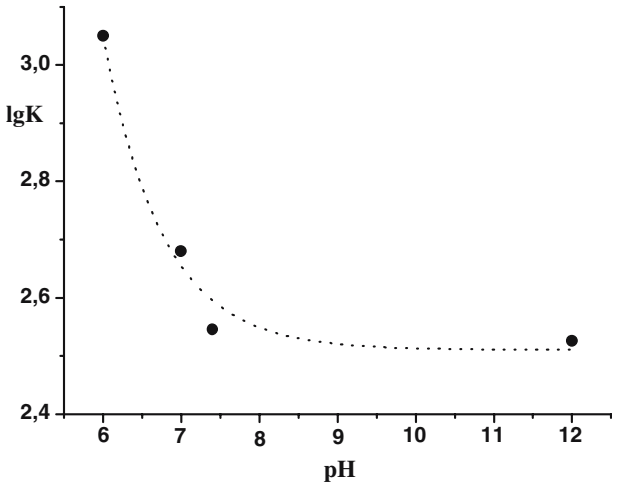

Figure 4. Plot the observed lg$K$ for complex formation of NA with β-CD against pH of the buffer solution.

Dependence of stability constants of the β-CD/NA complexes from pH is shown in Figure 4. The following tendency is observed: the value of lg$K$ is decreased at the pH enhancement. This dependence is in a good agreement with the regularity between the partial distribution of anionic form of the NA and pH-value (Figure 5). The plot presented on Figure 5 was obtained using the values of dissociation constants of NA in water $pK_1 = 2.26$ and $pK_2 = 4.44$ reported in Ref. [14]. As it is illustrated in Figure 5, the content of zwitterionic form of the NA is decreased while the content of anionic form is increased at pH range 3.5–14, and the anionic form becomes completely dominant at pH values above 7.2. As it follows from Figure 4, the zwitterionic and anionic forms show different binding abilities to binding with β-CD. β-CD forms more stable complex with zwitterions. The similar result was obtained in our previous work when we have studied the interaction of α-CD and HP-α-CD with cationic, anionic and zwitterionic forms of the nicotinic acid molecule [15].

It is well known, that the complexation of CDs with guest molecules occurs as the result of dehydration of

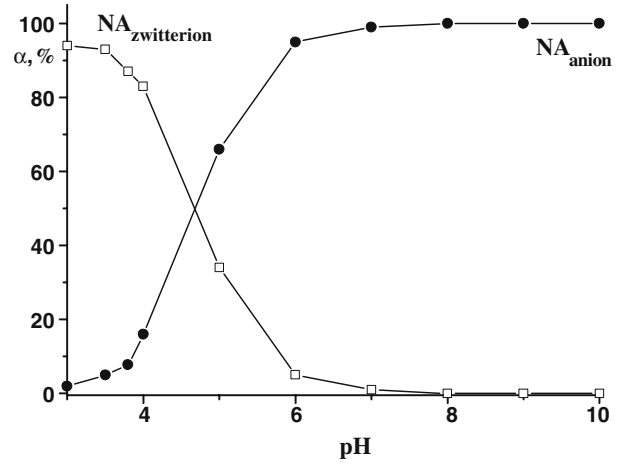

Figure 5. Distribution of NA species as function of pH.

reagents, release of cavity-bound high energy water, hydration of complex, direct binding due to non-covalent interactions (hydrophobic, van der Waals and electrostatic interactions, hydrogen bonding) and conformational changes [1–3]. It is sometimes supposed that inclusion of guest molecule into the CD cavity is driven by hydrophobic interactions. In this case the complex formation processes must be characterized by positive or small negative enthalpy changes and positive entropy changes [16–18]. However, as it follows from Table 2, the processes of NA complexation with the considered CDs are characterized by the relatively large and negative enthalpy and entropy changes. It means that the hydrophobic interactions do not play an essential role in complex formation. Probably, van der Waals interactions and H-bonding give a negative contribution to $\Delta_c H$ values. Moreover, the release of included in CDs cavities water molecules upon the complex formation with NA and hydration of complex are accompanied by negative enthalpy changes [19]. The negative entropy changes mean the enhancement of order in system during the complex formation process and can be attributed to stabilization of complexes by non-covalent interactions that are more important than solvent effects such as the release of water molecules from the cavity and the hydration sphere of reagents [20].

It was found, that the binding constants of NA complexes with different β-CDs can be arranged as: β-CD > HP-β-CD > M-β-CD. As respect to the native β-CD, the complexation of HP-β-CD with NA is characterized by a less negative enthalpy, the contribution from which decreases the stability constant and negative $\Delta_c G$ value. Thus, the presence of hydroxypropyl groups in β-CD molecule makes the complex formation process less enthalpically favorable. Decreasing exothermicity of binding may be a result of steric effect when the hydroxypropyl groups prevent the penetration of NA inside macrocyclic cavity. In this case binding is not so effective as with unsubstituted β-CD.

Methylation, as it is well known [21], makes the β-CD cavity more hydrophobic, increases the cavity depth and enhances flexibility of the macrocycle. From Table 2 it is not difficult to see, that M-β-CD forms less stable complex with the NA although this process is more enthalpically favorable ($\Delta_c H$ is more negative) due to better adaptation of NA to cavity. We assume, that deeper penetration of the NA molecule into M-β-CD cavity and, as consequence, a dominant role of van der Waals interactions as well as the exclusion of high-energy water from CD cavity give the negative contributions to $\Delta_c H$ value. Favorable enthalpy contribution to the free energy is compensated by the large negative (unfavorable) entropy term resulting in low stability constant and smallest $\Delta_c G$ value. The large negative entropy change reflects the fact that the NA loses a part of its degree of freedom during the complex formation with M-β-CD possessing deeper cavity. The possible H-bonding as

well as the van der Waals interactions result in formation of more ordered complexed species.

Conclusions

Interactions of NA with hydroxypropyl- and methyl-β-CDs as well as with native β-CD were studied by calorimetry, ^{1}H NMR spectroscopy and solubility method. The increase of NA solubility in aqueous HP-β-CD solutions was observed. The enhancement of solubility is explained by 1:1 complexation with HP-β-CD and by penetration of trifluoromethylphenyl residue of NA molecule into macrocyclic cavity. Therefore, β-CDs can be suitable candidates to resolve the problem of enhancement of solubility of poor water-soluble compounds.

Calculated thermodynamic parameters of NA complexation by native and modified β-CDs indicate that the binding is enthalpy driven. Stability of NA complexes with native β-CD is higher in comparison with substituted β-CDs studied. The determination of CDs inclusion complexes stability is of high importance because the processes of encapsulation with CDs, enhancement of solubility and stability of guest molecules and prolongation of therapeutic effect of drugs are based on the knowledge of binding constant values. Thus, chemically modified β-CDs are less effective hosts for the complex formation with NA than native β-CD. It is necessary to note that the nature of substitutes surrounding the macrocyclic cavity influences the thermodynamics of complex formation.

Acknowledgements

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