J Incl Phenom Macrocycl Chem (2007) 59:301–306
DOI 10.1007/s10847-007-9327-y

ORIGINAL ARTICLE

Inclusion complex formation of α- and β-cyclodextrins with aminobenzoic acids in aqueous solution studied by ^{1}H NMR

Irina V. Terekhova · Roman S. Kumeev · Gennadiy A. Alper

Received: 21 February 2007 / Accepted: 13 April 2007 / Published online: 7 June 2007

Abstract Complex formation of α- and β-cyclodextrins with *para*- and *meta*-aminobenzoic acids in water at 298.15 K was studied by ^{1}H NMR. The formation of 1:1 inclusion complexes in all systems under consideration was observed. Stability constants of the complexes and chemical shift difference induced by 100% complex formation were calculated. The obtained results were discussed in terms of influence of the cyclodextrin cavity size and position of the side amino group in the aromatic ring of aminobenzoic acid molecule on the binding mode and the complex stability.

Keywords Aminobenzoic acid · Binding mode · Cyclodextrin · Inclusion complexes · NMR · Stability constant

Introduction

Cyclodextrins (CDs) are an attractive candidates for encapsulating materials since they are the naturally occurring cyclooligosaccharides and, therefore, nontoxic. They possess an internal hydrophobic cavity in which different organic compounds can be included, while their hydrophilic external surface makes the host–guest complexes (or inclusion complexes) water soluble. Physical, chemical and biological properties of guest compounds can be considerably altered through the inclusion complex formation with CDs. Hence, the inclusion complexes of CDs found the numerous practical applications in pharmaceutical, cosmetic and food industries [1–4].

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

This work is a part of our systematic investigation of complex formation occurring between CDs and biologically active molecules in aqueous medium. Recently, we have studied the interactions of native and substituted α- and β-CD with some biologically active pyridine derivatives such as nicotinic acid, nicotinamide, pyridoxine and pyridoxal [5, 6]. As it was determined by different experimental methods, only nicotinic acid forms complexes with α-CD in aqueous solution at 298.15 K. Thus, it is interesting to continue the study on complex formation of CDs with aminobenzoic acids (ABA), in which the nitrogen atom occupies the absolutely different position in comparison with the nicotinic acid (3-pyridinecarboxylic acid).

Aminobenzoic acids are the benzoic acid derivatives having the amino group in *ortho*-, *meta*- or *para*-position of the aromatic ring. Among these structural isomers only *para*-aminobenzoic (pABA) is of biological importance. The pABA is known as vitamin H_1 belonging to B-group vitamins. pABA is widely distributed in nature, participates in some biochemical processes (e.g. biosynthesis of folic acid), and reduces the mutagenicity of chemical mutagens [7]. Moreover, pABA is used in the synthesis of some medicines (e.g. novocaine, 4-aminosalicylic acid) and as UV-filter in sunscreen cosmetics [8–10]. However, pABA can cause the possible unwanted side effects, which can limit its industrial applications. For instance, dermatological side effects and pABA capability to damage DNA after UV irradiation were observed [11–13]. To remove these unpleasant effects and to increase the aqueous solubility the using of ABA encapsulated forms on the basis of CDs can be proposed.

Analysis of the available in literature data showed, that the complex formation of α- and β-CD with pABA and *m*-aminobenzoic acid (mABA) have been studied using circular dichroism [14, 15], calorimetry [16], fluorescence [17], fluorimetry, NMR and UV–vis spectroscopy methods

[18–20]. Thermodynamic parameters of complex formation have been determined at different temperatures. Unfortunately, there are only a few publications [18–20], in which the binding of ABA with β-CD in DMSO-water mixtures has been investigated by ^{1}H NMR. As it was found [18, 19], β-CD forms with both mABA and pABA a 1:1 inclusion complexes in which the carboxyl group is located inside the hydrophobic cavity and amino group interacts with the hydrophilic part of the β-CD molecule. However, the possible interactions of organic cosolvent (DMSO) with the host can influence the binding mode and thermodynamics of complex formation of β-CD with ABA [19]. We have not found data regarding the structure of complexes formed between α-CD and ABA.

Therefore, this paper was devoted to study on complex formation of α- and β-CD with pABA and mABA in aqueous solution by means of ^{1}H NMR. The obtained in this work ^{1}H NMR data were used for (1) determination of the binding mode; (2) calculation of stability constants of the complexes; (3) analyzing of influence of the CD cavity size and position of the amino group in the ABA molecule on the binding mode and the thermodynamics of complex formation.

Experimental

Materials

p-Aminobenzoic acid (MP Biomedicals), *m*-aminobenzoic acid (MP Biomedicals), α-CD (Fluka), and β-CD (Fluka) were used as received. CDs were stable crystallohydrates, the water content in which determined by thermogravimetric analysis was 9% and 10% for α-CD and β-CD, respectively. The corrections for the water content were made during the calculation of the concentrations. All solutions were prepared by weight using the D_2O (99.9% of isotopic purity).

^{1}H NMR study

^{1}H NMR spectra were recorded at 200 MHz on Bruker AC-200 spectrometer. Temperature of 298.15 K was held constant (±0.10 K) using a Bruker BVT-3000 temperature control system. All chemical shifts were measured relative to external cyclohexane.

The stoichiometry of the complexes was determined by the continuous variation method (Job method) [21]. According to this method, CD and ABA solutions of equal initial concentrations (0.01 mol kg^{-1}) were mixed to constant volume keeping the overall concentration of ABA and CD constant ([CD] + [ABA] = 0.01 mol kg^{-1}) and varying the ratio R (R = [CD]/([CD] + [ABA])) from 0 to 1. The stoichiometry can be obtained by plotting $\Delta\delta_{CD}\cdot R$ against R and finding R corresponding to the extremum of these distributions.

To determine the stability constant of the complexes the ^{1}H NMR titration was performed. The initial concentration of ABA was fixed (0.005 mol kg^{-1}), whereas the CD concentration was varied.

Results and discussion

Inclusion of the ABA in the CD molecule can be shown by the induced by complex formation changes in the chemical shifts of both host and guest. Therefore, to obtain the detail information on the binding mode of α- and β-CD with ABA consideration of the proton shifts induced by 100% complexation ($\Delta\delta_c$) together with the thermodynamic parameters of complex formation will be useful.

For this purpose the stability constants (K) of the complexes were calculated for 1:1 binding that is described by the Equations:

$$CD + ABA = CD\cdot ABA \quad (1)$$

$$K = [CD\cdot ABA]/([CD]\cdot[ABA]) \quad (2)$$

For all systems under study the 1:1 stoichiometry of the complexes was demonstrated in continuous variation plots (Fig. 1). The maximum of curves shown in Fig. 1 corresponds to R = 0.5, confirming the existence of complexes of single 1:1 composition within the range of concentrations studied.

The total concentrations of CD and ABA are

$$C_{CD} = [CD] + [CD\cdot ABA] \quad (3)$$

$$C_{ABA} = [ABA] + [CD\cdot ABA] \quad (4)$$

Thus stability constant is

$$K = [CD\cdot ABA]/((C_{CD} - [CD\cdot ABA])\cdot(C_{ABA} - [CD\cdot ABA])) \quad (5)$$

The observed chemical shift (δ_{exp}) can be written as follows

$$\delta_{exp} = \delta_{ABA}\cdot([ABA]/C_{ABA}) + \delta_{CD\cdot ABA}\cdot([CD\cdot ABA]/[C_{ABA}]) \quad (6)$$

Eq. 6 can be transformed into

$$(\delta_{exp} - \delta_{ABA})/(\delta_{CD\cdot ABA} - \delta_{ABA}) = [CD\cdot ABA]/C_{ABA} \quad (7)$$

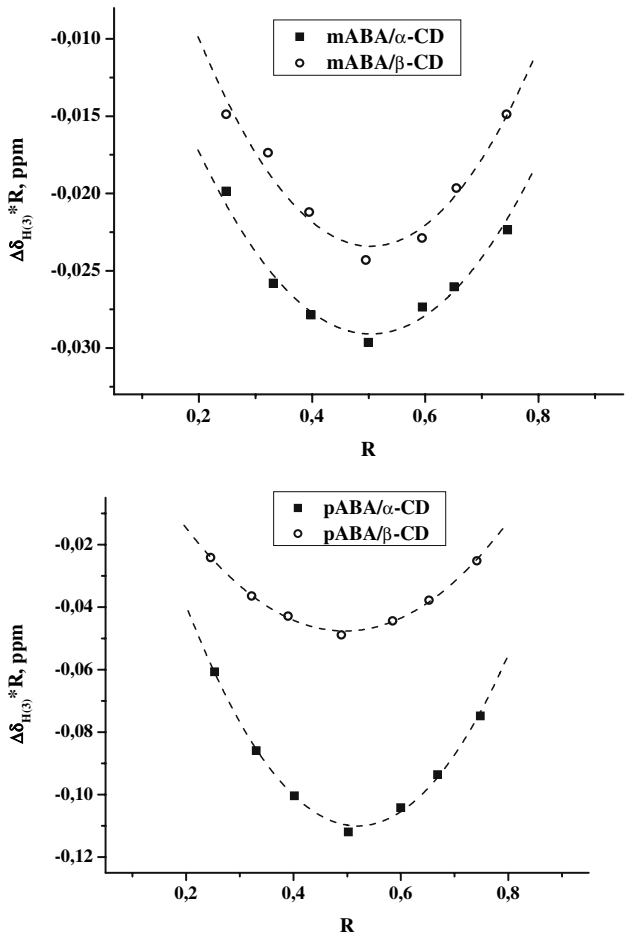


Fig. 1 Continuous variation plots for complexes of aminobenzoic acids with CDs

Letting $\Delta\delta = \delta_{exp} - \delta_{ABA}$ and $\Delta\delta_c = \delta_{CD \cdot ABA} - \delta_{ABA}$, Eq. 7 can be rewritten as

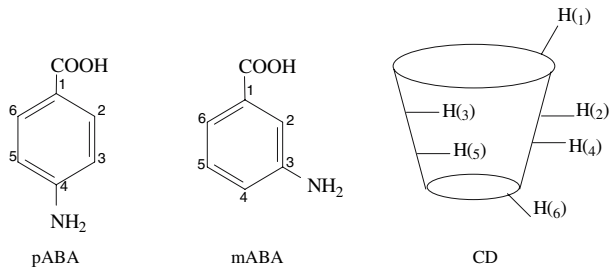
$$\Delta\delta/\Delta\delta_c = [CD \cdot ABA]/C_{ABA} \quad (8)$$

Combination of Eq. 8 with Eq. 5 gives

$$K = C_{ABA} \cdot \Delta\delta/(\Delta\delta_c \cdot (C_{CD} - C_{ABA} \cdot \Delta\delta/\Delta\delta_c) \cdot (C_{ABA} - C_{ABA} \cdot \Delta\delta/\Delta\delta_c)) \quad (9)$$

Analytical solution of Eq. 9 by non-linear regression analysis gives K and $\Delta\delta_c$, the values of which are listed in Table 1. As an example, Fig. 2 illustrates the best fit curves for H(2) and H(4) protons of pABA complexing by β-CD. A good agreement between K values calculated in this work (Table 1) and published in literature (Table 2) was noted.

The results summarized in Table 1 reveal that complex formation of α-CD with mABA and pABA induces the downfield shift for the ABA H(2) and H(6) protons which are placed close to the carboxylic group (Scheme 1). It suggests that penetration of pABA and mABA into α-CD cavity is shallow. The carboxylic group is located inside the cavity, whereas the amino group resides within the polar exterior of α-CD molecule. To confirm this fact the consideration of α-CD ^{1}H NMR spectrum is necessary.



Scheme 1 Objects under study. Schematic presentation of the structure and atom numbering

Figure 3 shows the partial ^{1}H NMR spectra of α-CD with and without mABA and pABA. The ^{1}H NMR spectrum of CD consists of the signals of H(1), H(2), H(3), H(4), H(5) and H(6) protons (Scheme 1). It is necessary to note, that H(1), H(2), H(4) and H(6) protons are located on the exterior of CD molecule. To the contrary, H(3) and H(5) protons are located inside the macrocyclic cavity at the wider and narrow rims, respectively, and they are most sensitive to the inclusion process [22]. As can be seen from Fig. 3, addition of pABA induces the pronounced upfield shift for H(3) proton ($\Delta\delta_c = -0.32 \pm 0.01$ ppm) and downfield shift for H(5) proton ($\Delta\delta_c = 0.19 \pm 0.01$ ppm). The negligible upfield shift for H(1), H(2), H(4) and H(6) protons was also observed. The influence of the mABA on the α-CD ^{1}H NMR spectrum is analogous (Fig. 3). The ''external'' protons are practically unaffected in the presence of mABA, whereas the signals of the ''internal'' H(3) and H(5) protons exhibit an upfield ($\Delta\delta_c = -0.35 \pm 0.01$ ppm) and downfield ($\Delta\delta_c = 0.16 \pm 0.01$ ppm) shift, respectively. The magnitude and sing of $\Delta\delta_c$ for the α-CD H(3) and H(5) protons indicate that pABA and mABA are inserted into macrocyclic cavity predominantly from the wider rim of α-CD molecule. The depth of penetration is approximately the same for both acids under study. The location of the ABA aromatic ring inside the macrocyclic cavity induces the observed shielding of the α-CD H(3) proton. The carboxylic group positioned near the α-CD H(5) protons causes their deshielding. The similar results were found for complex formation of α-CD with some substituted benzoic acids [23] and phenyl derivatives [24].

The signals of all protons of the ABA aromatic ring are upfield shifted upon complex formation with β-CD (Table 1). It indicates that the deeper insertion of pABA and mABA into β-CD cavity takes place. Figure 4 shows

Table 1 Apparent stability constants and ^{1}H NMR chemical shift difference for aminobenzoic acid protons induced by 100% complex formation with α- and β-CDs in aqueous solution at 298.15 K

Complex	lg K	$\Delta\delta_c$ ppm				
		H(2)	H(3)	H(4)	H(5)	H(6)
mABA/α-CD	1.8 ± 0.4	0.09 ± 0.02	–	~0	~0	0.09 ± 0.02
pABA/α-CD	3.1 ± 0.6	0.32 ± 0.01	~0	–	~0	0.32 ± 0.01
mABA/β-CD	1.8 ± 0.3	–0.33 ± 0.04	–	–0.17 ± 0.07	–0.14 ± 0.05	–0.36 ± 0.06
pABA/β-CD	2.8 ± 0.6	–0.09 ± 0.01	–0.13 ± 0.01	–	–0.13 ± 0.01	–0.09 ± 0.01

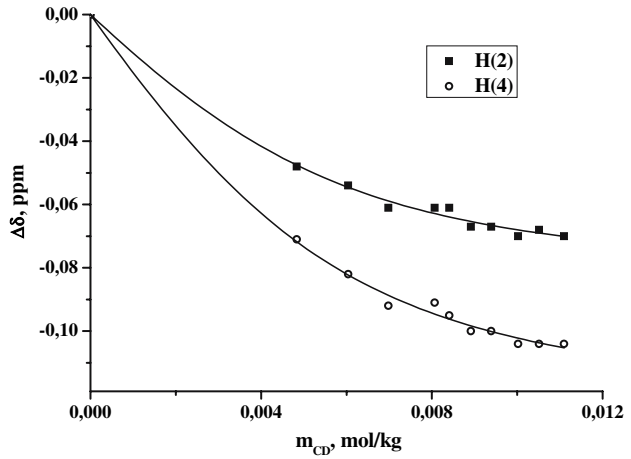


Fig. 2 Chemical shift changes of pABA protons plotted against the β-CD concentration (298.15 K)

the partial ^{1}H NMR spectra of β-CD alone and in the presence of pABA and mABA. It is not difficult to see that addition of both acids induces the upfield shift for H(3) and H(5) protons, the shifting of the last being more sizeable. For pABA complexes, the values of $\Delta\delta_c = -0.18 \pm 0.01$ ppm and $\Delta\delta_c = -0.24 \pm 0.01$ ppm were obtained for H(3) and H(5), respectively. For mABA complexes, the following $\Delta\delta_c$ were estimated: $\Delta\delta_c = -0.19 \pm 0.01$ ppm and $\Delta\delta_c = -0.23 \pm 0.01$ ppm for H(3) and H(5), respectively. The measurable upfield shifts obtained for both H(3) and H(5) protons demonstrate that the aromatic ring of pABA and mABA is deeply included into the β-CD cavity and located between H(3) and H(5) planes. However, it is not possible to indicate from which side of the β-CD cavity the aromatic ring is inserted.

Thus, it was revealed that CD cavity dimensions play an important role in complex formation with mABA and pABA and determine the depth of penetration. β-CDs possessing the larger cavity diameter are more suitable for the inclusion of ABA in aqueous solution. On the contrary, the position of the side NH_2-group in the ABA molecule has no influence on the inclusion depth and the binding mode with α- and β-CD. However, the stability of the complexes and the other thermodynamic parameters of complex formation are sensitive to placement of NH_2-group in the benzene ring. In comparison with mABA, the binding of pABA with α- and β-CD is accompanied by more exothermic enthalpy changes and results in formation of more stable complexes (Tables 1 and 2). It should be also mentioned, that within the each ABA the CD cavity size influences only the enthalpy and entropy terms, while the stability constant values remain independent on the cavity diameter (Tables 1 and 2).

As can be seen from Table 2, the complex formation of α-CD with pABA and mABA is characterized by more negative enthalpy and entropy changes (Table 2). Probably, dispersive interactions and H-bonding are the main source of the negative Δ_cH and Δ_cS values. The binding of α-CD with pABA is more enthalpically favorable and results in formation of more structured and stable complexed species. The lower Δ_cH and Δ_cS values obtained for pABA complexes can be additionally caused by the possible participation of NH_2-group placed in the *para*-position in the hydrogen bonding with OH-groups surrounding the macrocyclic cavity.

The deeper insertion of pABA and mABA into β-CD cavity that occurs according to the obtained ^{1}H NMR data

Table 2 Literature data on thermodynamics of complex formation of CDs with ABA in aqueous solution at 298 K

Complex	lg K	Δ_cG^a	Δ_cH^a	$T\Delta_cS^a$	Method	Ref.
mABA/α-CD	1.7	–9.9	–32.5 ± 0.3	–22.6 ± 0.8	Circular dichroism	[15]
pABA/α-CD	2.8 ± 0.1	–15.9 ± 0.4	–49 ± 2	–33	Calorimetry	[16]
	2.8	–16.0	–43.6 ± 0.8	–27.6 ± 2.5	Circular dichroism	[15]
mABA/β-CD	1.8	–10.3	–8.7 ± 0.3	1.6 ± 1.3	Circular dichroism	[15]
pABA/β-CD	2.7	–15.4	–23.4 ± 0.4	–8.0 ± 1.3	Circular dichroism	[15]

[a] Units: kJ mol^{-1}

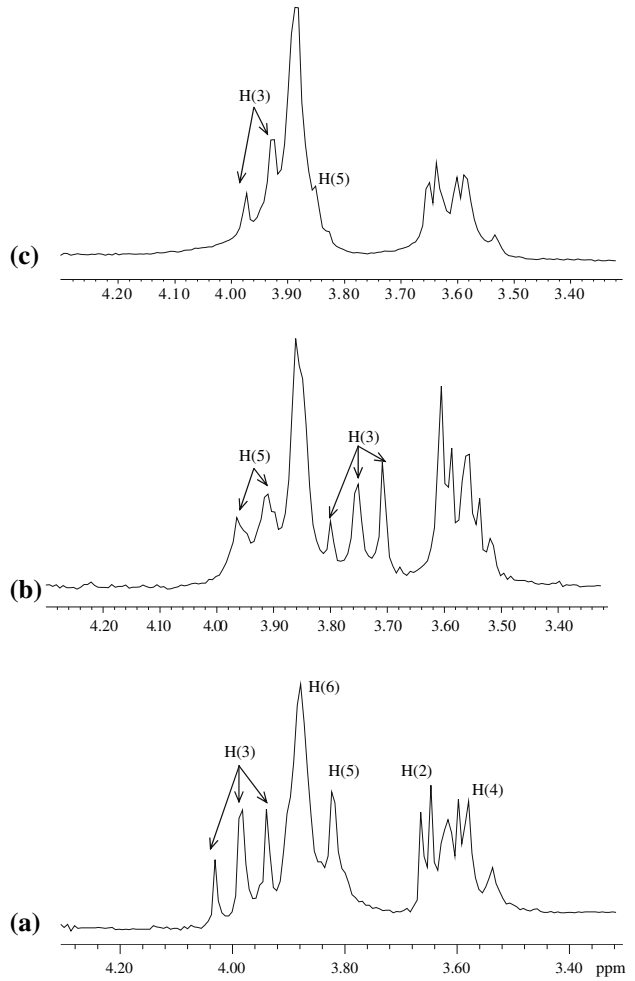


Fig. 3 Partial ^{1}H NMR spectra (200 MHz, 298.15 K) of 5 mM α-CD alone (**a**) and in the presence of 5 mM pABA (**b**) and 5 mM mABA (**c**)

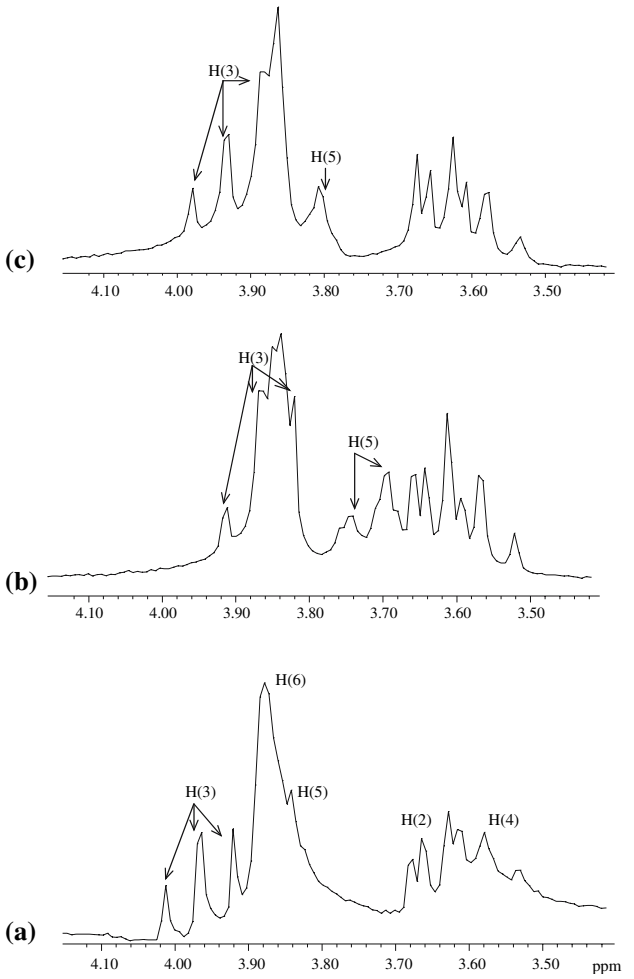


Fig. 4 Partial ^{1}H NMR spectra (200 MHz, 298.15 K) of 5 mM β-CD alone (**a**) and in the presence of 5 mM pABA (**b**) and 5 mM mABA (**c**)

promotes the release of water molecules from the outer solvation shells of reagents and CD cavity in the bulk aqueous solution. The endothermic contributions from dehydration and hydrophobic interactions arisen between hydrophobic cavity and ABA aromatic ring result in a considerable decrease of the exothermicity of binding of β-CD with both acids under study. Thus, the proposed modes of CD-ABA binding are in agreement with the thermodynamic parameters of complex formation.

Conclusions

Complex formation of α- and β-CD with isomeric mABA and pABA in aqueous solution at 298.15 K was studied by ^{1}H NMR. As it was obtained, the depth of ABA penetration into the CD cavity is mainly determined by the cavity size. Insertion of pABA and mABA into α-CD cavity is shallow and is governed predominantly by the van der Waals interactions and H-bonding. To the contrary, the deeper inclusion of pABA and mABA into β-CD cavity is accompanied by the intensive dehydration and hydrophobic effects. Thus, β-CDs were found as more suitable macrocycles for encapsulation of ABA. Position of the side amino group in the aromatic ring of ABA has no noticeable influence on the binding mode with α- and β-CD, whereas its influence on the complex stability and thermodynamic parameters of complex formation was observed. In comparison with mABA, pABA forms with α- and β-CD more stable and enthalpy stabilized complexes.

Acknowledgements This work was supported by the Russian Science Support Foundation. We would like also to thank N.A. Obukhova for her assistance in the experimental part of work.

References

1. Szejtli, J.: Cyclodextrin Technology, Kluwer Academic Publishers, Netherlands (1988).

2. Szejtli, J.: Industrial applications of cyclodextrins. In: Atwood, J.L., Davies, J.E.D., MacNicol, D.D. (eds.) Inclusion Compounds, vol. 3. Academic Press, London (1984).
3. Hedges A.R.: Industrial applications of cyclodextrins. Chem. Rev. **98**, 2035–2044 (1998).
4. Uekama, K., Hirayama, F., Irie, T.: Cyclodextrin drug carrier systems. Chem. Rev. **98**, 2045–2076 (1998).
5. Terekhova, I.V., Kulikov, O.V.: Cyclodextrins. Physical–chemical aspects of formation of complexes ''host–guest'' and molecular selectivity in relation to biologically active molecules.In: Zaikov, G.E. (ed.) Chemistry of Polysaccharides. Brill Academic Publishers, Netherlands (2005).
6. Terekhova, I.V., Scriba, G.K.E.: Study on complex formation of biologically active pyridine derivatives with cyclodextrins by capillary electrophoresis. J. Pharm. Biomed. Anal. (2007) doi: 10.1016/j.jpba.2007.02.003.
7. Vasilieva, S.: Para-aminobenzoic acid inhibits a set of SOS functions in Escherichia coliK12. Mutat. Res. **496**, 89–95 (2001).
8. Hirt, R., Hurni, H.: Helv. Chim. Acta **32**, 378 (1949).
9. Bruze, M., Gruvberger, B., Thulin I.: PABA, benzocaine, and other PABA esters in sunscreens and after-sun products. Photodermatol. Photoimmunol. Photomed. **7**, 106–108 (1990).
10. Mackie, B.S., Mackie, L.E.: The PABA story. Aust. J. Dermatol. **40**, 51–53 (1999).
11. Salvador, A., Chisvert, A., Rodríguez, A., March, J.G.: Indirect spectrophotometric determination of p-aminobenzoic acid in sunscreen formulations by sequential injection analysis. Anal. Chem. Acta **493**, 233–239 (2003).
12. Dromgoole, S.H., Mailbach, H.I.: Sunscreening agent intolerance: contact and photocontact sensitization and contact urticaria. J. Am. Acad. Dermatol. **22**, 1068–1078 (1990).
13. Shaw, A.A., Wainschel, L.A., Shetlar, M.D.: Photoaddition of p-aminobenzoic acid to thymine and thymidine. Photochem. Photobiol. **55**, 657–663(1992).
14. Setnička, V., Urbanová, M., Král, V., Volka, K.: Interactions of cyclodextrins with aromatic compounds studied by vibrational circular dichroism spectroscopy. Spectrochim. Acta A **58**, 2983–2989(2002).
15. Harata, K.: Induced circular dichroism of cycloamylose complexes with meta-and para-disubstituted benzenes. Bioorg. Chem. **10**, 255–265 (1981).
16. Lewis, E.A., Hansen, L.D.: Thermodynamics of binding of guest molecules to α- and β-cyclodextrins. J. Chem. Soc. Perkin Trans. **2**, 2081–2085(1973).
17. Shaomin, S., Yu, Y., Jinghao, P.: Study on molecular recognition of para-aminobenzoic acid species by α-, β- and hydroxypropyl-β-cyclodextrin. Anal. Chim. Acta **458**, 305–310 (2002).
18. Stalin, T., Rajendiran, N.: Intramolecular charge transfer effects on 3-aminobenzoic acid. Chem. Phys. **322**, 311–322 (2006).
19. Stalin, T., Shanthi, B., Vasantha Rani, P., Rajendiran, N.: Solvatochroism, prototropism and complexation of p-aminobenzoic acid. J. Inclus. Phenom. Macrocycl. Chem. **55**, 21–29 (2006).
20. Stalin, T., Rajendiran, N.: Intramolecular charge transfer associated with hydrogen bonding effects on 2-aminobenzoic acid. J. Photochem. Photobiol. A **182**, 137–150 (2006).
21. Job, P.: Ann. Chim. **9**, 113–203 (1928).
22. Schneider, H.-J., Hacket, F., Rűdiger, V.: NMR studies of cyclodextrins and cyclodextrin complexes. Chem. Rev. **98**, 1755–1785(1998).
23. Simova, S., Schneider, H.-J.: NMR analyses of cyclodextrin complexes with substituted benzoic acids and benzoate anions. J. Chem. Soc. Perkin Trans. **2**, 1717–1722 (2000).
24. Rűdiger, V., Eliseev, A., Simova, S., Schneider, H.-J., Blandamer, M.J., Cullis, P.M., Meyer, A.J.: Conformational, calorimetric and NMR spectroscopic studies on inclusion complexes of cyclodextrins with substituted phenyl and adamantine derivatives. J. Chem. Soc. Perkin Trans. **2**, 2119– 2123 (1996).