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## IR Spectroscopy, X-Ray Diffraction and Thermal Analysis Studies of Solid “ $\beta$ -Cyclodextrin - *Para*-Aminobenzoic Acid” Inclusion Complex

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Interactions between components of solid “ $\beta$ -cyclodextrin – *para*-aminobenzoic acid” inclusion complex were confirmed by combining the IR spectroscopy data with the results of X-ray diffraction and thermal analysis (DTA, TG, DTG). It was established that the vibrations and bends of the “guest” molecule are restricted through encapsulation of *para*-aminobenzoic acid into the  $\beta$ -cyclodextrin cavity. The degree of crystallinity of supramolecular complex substantially decreases in comparison with  $\beta$ -cyclodextrin and *para*-aminobenzoic acid. The enhancement of thermal stability for included *para*-aminobenzoic acid was observed. It was found a decrease of water content in the inside cavity of  $\beta$ -cyclodextrin and an increase of activation energy of dehydration as a result of supramolecular compound formation.

**Key words:**  $\beta$ -cyclodextrin, *para*-aminobenzoic acid, IR spectroscopy, thermal analysis, X-ray diffraction.

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### Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides which compose of several D-glucose units linked by  $\alpha(1\rightarrow4)$  bonds. These compounds form cone-shaped molecules with primary hydroxyl groups arranged in an inner hydrophobic cavity and secondary hydroxyl groups rendering external walls hydrophilic. Thus, CDs are both lipophilic and soluble in water. They as molecular “hosts” form inclusion complexes with a wide variety of compounds – from very polar inorganic ions to completely nonpolar organic molecules. Partially or entirely encapsulation of “guests” occurs if their molecular dimensions correspond to those of cyclodextrin cavity. The driving forces leading to the inclusion complex formation include electrostatic interactions, van der Waals interactions, hydrophobic interactions, hydrogen bonding, release of conformational strain, exclusion of cavity-bound “high-enthalpy” water and charge-transfer interactions [1]. In recent years, complex formation with CDs has been successfully used to improve solubility, chemical stability and bioavailability, to decrease toxicity and to control releasing of biologically active compounds.

In our recent work [2], protolytic and complex formation properties of *para*-aminobenzoic acid (*p*-ABA), well known antioxidant, antimutagene and antitumor agent [3 – 5], in the presence of  $\beta$ -CD were

studied in buffer solutions by UV spectroscopy. The stoichiometry and stability constant of “ $\beta$ -CD – *p*-ABA” supramolecular complex were determined by the Ketelar equation [6]. Calculation of the thermodynamic parameters involved in the complex formation were realized by use of the van't Hoff equation. It was proved that complex formation between *p*-ABA and  $\beta$ -CD is spontaneous exothermic process resulting in the decreasing of system entropy. In this work synthesized solid inclusion complex “ $\beta$ -CD – *p*-ABA” with stoichiometric ratio of components  $\beta$ -CD : *p*-ABA = 1 : 1 has been studied by IR spectroscopy, X-ray diffraction and thermal analysis.

### I. Experimental

#### 1.1. Materials and chemicals.

$\beta$ -CD (from Fluka, purity  $\geq 99\%$ ) and *p*-ABA (from Merk, purity  $\geq 99\%$ ) were used as received (without additional purification).

#### 1.2. Preparation of solid inclusion complex of *p*-ABA with $\beta$ -CD.

Batches of  $\beta$ -CD and *p*-ABA were weighed in a 1 : 1 molar ratio and dissolved singly in twice-distilled water. Then the  $\beta$ -CD solution was gradually dropped to the *p*-ABA solution and continuously agitated with an

electromagnetic stirrer at 20 °C for 24 h. The binary solution was stored at 4 °C for 48 h. The precipitate was washed with twice-distilled water. After drying in an oven at 60 °C for 24 h, a white powdered product was obtained.

### 1.3. Methods and instruments.

X - ray diffraction spectra of  $\beta$ -CD, *p*-ABA and “ $\beta$ -CD – *p*-ABA” inclusion complex were registered by use of a diffractometer DRON - 4 - 07 (emission of  $\text{CuK}\alpha$ ,  $\lambda = 1,54178 \text{ \AA}$ ) with nickel filter.

IR spectra of  $\beta$ -CD, *p*-ABA and “ $\beta$ -CD – *p*-ABA” inclusion complex were recorded in the wavenumber range from 4000 to 1200  $\text{cm}^{-1}$  with a Thermo Nicolet NEXUS FT-IR spectrophotometer using KBr pelleting.

Thermal analysis was performed on a computer automated equipment for the simultaneous TG and DTA measurements – Derivatograph-C (Paulic-Paulic-Erdey). Samples were heated from 30 to 900 °C in open ceramic crucibles in static air atmosphere, using  $\text{Al}_2\text{O}_3$  as an inert standard. Heating rate was 10 °C·min<sup>-1</sup>, and batch was 100 mg.

## II. Results and discussion

### 2.1. IR spectral study of *p*-ABA, $\beta$ -CD and “ $\beta$ -CD – *p*-ABA” inclusion complex.

Inclusion complex formation may be confirmed by IR spectroscopy because bands resulting from the included “guest” molecule are generally shifted or their intensities are altered.

In the IR spectrum of *p*-ABA (Fig. 1, curve 1) the valence vibrations of the N–H bonds in the primary amino group and the C–H bonds in the aromatic ring with maxima at 3460, 3362, 3231  $\text{cm}^{-1}$  and 3051  $\text{cm}^{-1}$ , respectively, are registered. The band of valence vibrations of the C=O bond in the carboxyl group is observed at 1661  $\text{cm}^{-1}$ . The absorption bands with maxima at 1600, 1573 and 1524  $\text{cm}^{-1}$  belong to the valence vibrations of the C=C bonds in the benzene ring. The valence vibrations of the C–N bond in the amino group connected with benzene ring are observed at 1310  $\text{cm}^{-1}$ . The bands of the deformation vibrations of the N–H bonds in the amino group and the C–H bonds in the benzene ring are registered at 1625, 890  $\text{cm}^{-1}$  and 1173, 1131, 844  $\text{cm}^{-1}$ , respectively [7].

In the IR spectrum of  $\beta$ -CD (Fig. 1, curve 2) the wide band is registered with the absorption maximum at 3320  $\text{cm}^{-1}$ , which is caused by the valence vibrations of the O–H bonds in the primary hydroxyl groups (C – 6 - OH) connected by the intermolecular hydrogen bonds or in the secondary hydroxyl groups connected by the intramolecular hydrogen bonds (the C – 2 - OH group of one glucopyranose unit and C – 3 - OH group of the adjacent glucopyranose unit) [8]. Also, in the IR spectrum of  $\beta$ -CD the absorption band with maximum at 2926  $\text{cm}^{-1}$  is observed. It belongs to the valence vibrations of the C–H bonds in the CH and CH<sub>2</sub> groups.

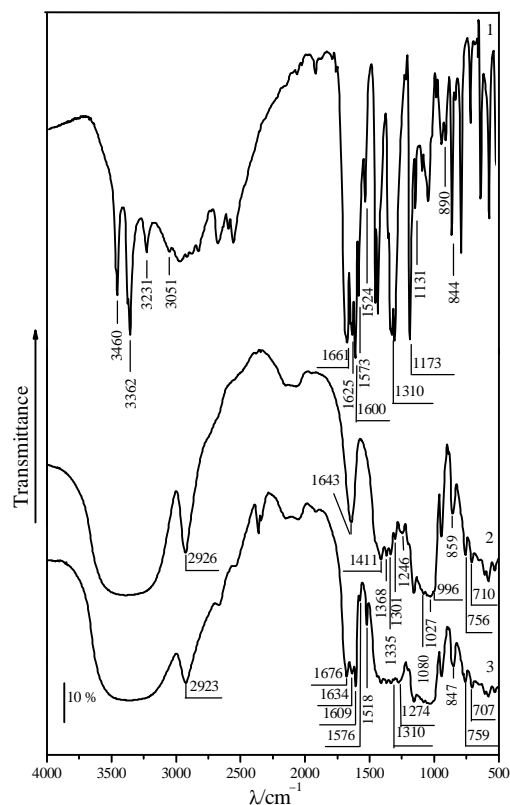


Fig. 1. IR spectra of *p*-ABA (1),  $\beta$ -CD (2) and “ $\beta$ -CD – *p*-ABA” inclusion complex (3).

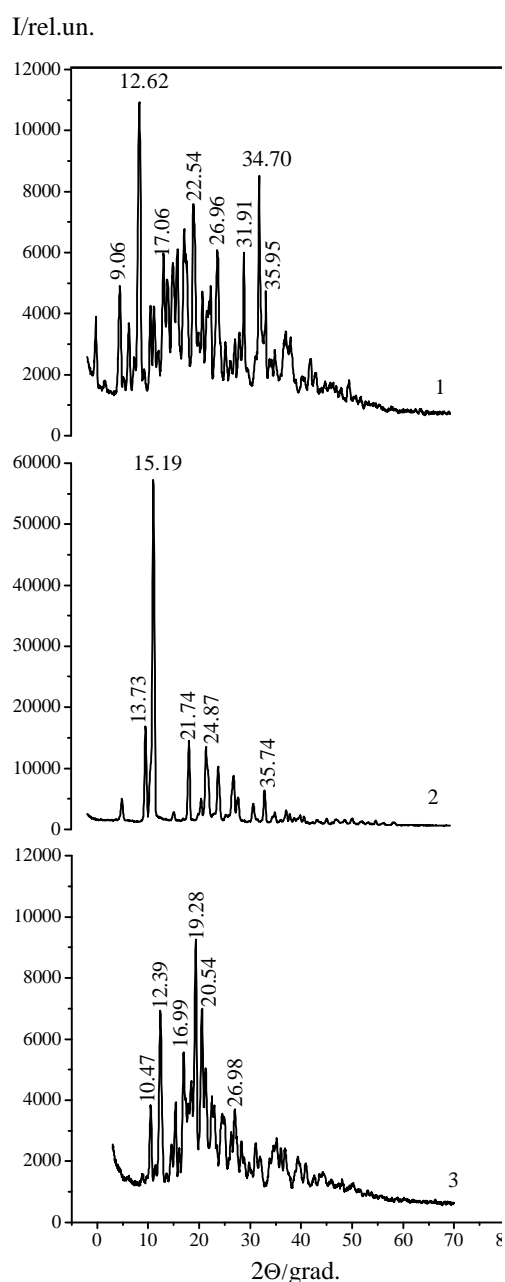
In the region 1400 – 1200  $\text{cm}^{-1}$  the absorption bands of the deformation vibrations of the C–H bonds in the primary and secondary hydroxyl groups of  $\beta$ -CD (1411, 1368, 1335, 1301, 1246  $\text{cm}^{-1}$ ), and in the interval 1200–1030  $\text{cm}^{-1}$  the absorption bands of the valence vibrations of the C–O bonds in the ether and hydroxyl groups of  $\beta$ -CD (1080 and 1027  $\text{cm}^{-1}$ ) are registered. The absorption bands in the region 950 – 700  $\text{cm}^{-1}$  belong to the deformation vibrations of the C–H bonds and the pulsation vibrations in glucopyranose cycle.

The IR spectrum of the inclusion complex “ $\beta$ -CD – *p*-ABA” differs from the IR spectra of *p*-ABA and  $\beta$ -CD (Fig. 1, curve 3). The band of the valence vibrations of the C=O bond in the carboxyl group of *p*-ABA is shifted to higher wavenumber in spectral pattern of the inclusion complex and registered at 1676  $\text{cm}^{-1}$ . At the same time, the band of the deformation vibrations of the N–H bonds in the amino group is shifted to lower wavenumber and observed at 1634  $\text{cm}^{-1}$ . The absorption bands of the valence vibrations of the C–O bonds in the ether and hydroxyl groups of  $\beta$ -CD in the interval 1200 – 1030  $\text{cm}^{-1}$  are slightly broadened for the inclusion complex. Moreover, the absorption bands of the valence vibrations of the C=C bonds in the benzene ring are shifted to 1609, 1576 and 1518  $\text{cm}^{-1}$ ; the peak at 1246  $\text{cm}^{-1}$  in the spectrum of  $\beta$ -CD which belongs to the deformation vibrations of the C–H bonds in the hydroxyl groups is shifted to the 1274  $\text{cm}^{-1}$  and greatly broadened. In the IR spectrum of the inclusion complex (Fig. 1, curve 3), maxima of characteristic

absorption bands of glucopyranose unit are assigned to 847, 759 and 707  $\text{cm}^{-1}$ . These results indicate that the vibrations and bends of the "guest" molecule are restricted through encapsulation of *p*-ABA into the  $\beta$ -CD cavity and formation of the inclusion complex.

## 2.2. X-ray diffraction study of *p*-ABA, $\beta$ -CD and " $\beta$ -CD – *p*-ABA" inclusion complex.

Figure 2 shows the X-ray powder diffraction patterns of  $\beta$ -CD, *p*-ABA and " $\beta$ -CD – *p*-ABA" inclusion complex obtained by the coprecipitation from the water binary solution with molar ratio of  $\beta$ -CD : *p*-ABA = 1 : 1. Diffractograms of the inclusion complex and initial compounds differ markedly. Sharp peaks at diffraction angles of 13,73, 15,19, and 21,74  $2\theta$  are present in the diffractogram of *p*-ABA (Fig. 2).

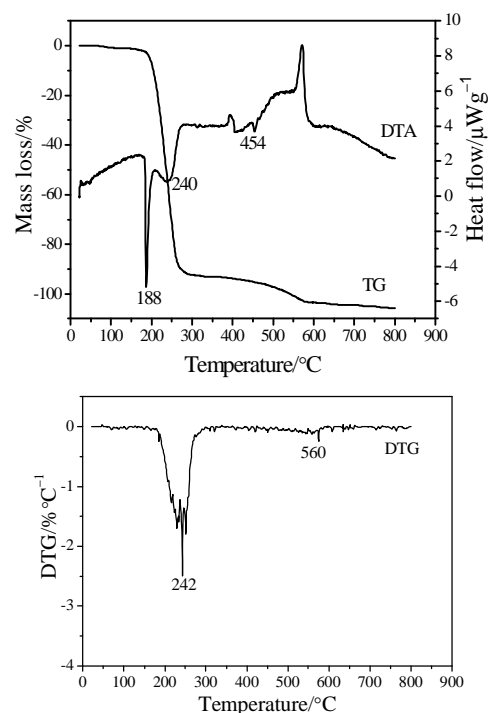


**Fig. 2.** Diffractograms of  $\beta$ -CD (1), *p*-ABA (2) and " $\beta$ -CD – *p*-ABA" inclusion complex (3).

Diffractogram of  $\beta$ -CD contains peaks at diffraction angles of 12,62, 22,54, and 34,70  $2\theta$ . X-ray diffraction pattern of " $\beta$ -CD – *p*-ABA" supramolecular complex is characterized by diffraction peaks in which it is no longer possible to distinguish the characteristic peaks of *p*-ABA. Encapsulation of „guest" molecule into the cavity of  $\beta$ -CD results in the appearance of new diffraction peaks at diffraction angles of 12,62, 22,54, and 34,70  $2\theta$ . Their intensities are commensurable with the intensities of individual components or higher. Interaction between *p*-ABA and  $\beta$ -CD changes the crystalline structure of  $\beta$ -CD. The substantial decrease of inclusion complex crystallinity in comparison with  $\beta$ -CD and *p*-ABA and appearance of amorphous halo are the result of „guest" molecule incorporation into the inner cavity of the „host" compound. In accordance with the data obtained by the single-crystal X-ray diffraction [9] location of amino and carboxyl groups of *p*-ABA on the wide and narrow edge of the  $\beta$ -CD molecule, respectively, is the most likely.

## 2.3. Thermal analysis of *p*-ABA, $\beta$ -CD and " $\beta$ -CD – *p*-ABA" inclusion complex.

Thermal destruction of the solid " $\beta$ -CD – *p*-ABA" inclusion complex has been studied. Figure 3 demonstrates the results of thermal analysis of *p*-ABA. On the DTA curve of *p*-ABA thermic effects of water molecules removal are absent, and pronounced endothermic effect of melting process without mass loss on the TG and DTG curves is registered. The experimental melting point at  $T_{\text{max}} = 188$  °C is in a good agreement with literature data for *p*-ABA ( $T_{\text{melt}} = 187$ – $189$  °C) [10]. Consequently, studied *p*-ABA is crystalline nonhydrated compound without any impurities. Thermal destruction of *p*-ABA melt above 200 °C proceeds in two stages. At first stage mass loss in



**Fig. 3.** Thermal analysis of *p*-ABA.

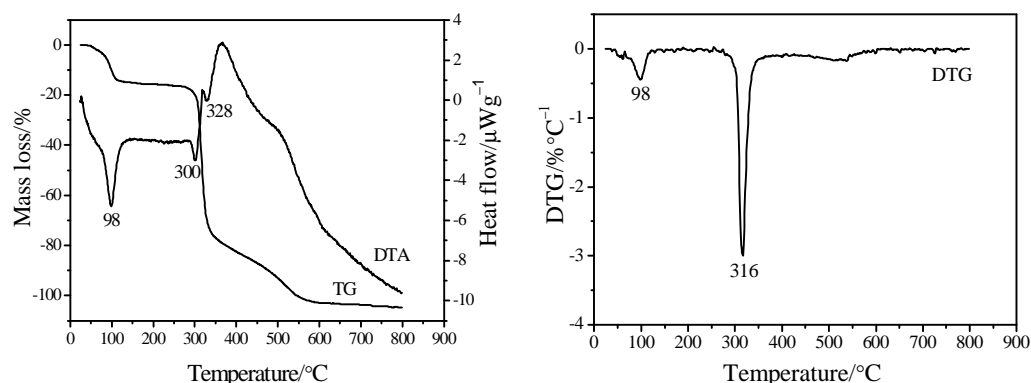


Fig. 4. Thermal analysis of  $\beta$ -CD.

the temperature range from 200 to 300 °C (endothermic effect at  $T_{\max} = 240$  °C) is equal to 90 %. Mass loss of the second stage (from 400 to 600 °C) equals 10 % of initial batch of *p*-ABA. Thermal destruction of benzoic acid proceeds in the similar way, except that it is finished about 300 °C. Hence, one can concede that in the temperature range from 400 to 600 °C thermal destruction of amino-containing fragments of *p*-ABA occurs. Obtained results are in a good agreement with literature data, for example, thermal destruction of aminopropyl radicals takes place at 500 – 540 °C [11].

As shown in thermogram of  $\beta$ -CD (Figure 4), there are five thermic effects with mass loss. Low-temperature endothermic effect at  $T_{\max} = 98$  °C belongs to the release of water molecules from the inner cavity of  $\beta$ -CD. Endothermic effects at  $T_{\max} = 300$  and 328 °C correspond to the destruction of hydroxyl groups of  $\beta$ -CD, and exothermic effect with two maxima at  $T_{\max} = 375$  and 500 °C relates to decomposition of glucopyranose units.

As can be seen from Figure 5, for “ $\beta$ -CD – *p*-ABA” inclusion complex the endothermic effect of water molecules removal from the inner cavity of  $\beta$ -CD is shifted to  $T_{\max} = 115$  °C. There are no endothermic effects of melting and breaking down of *p*-ABA in thermogram of inclusion complex. At the same time, characteristic thermic effects of  $\beta$ -CD are registered. The mass loss of inclusion compound in the temperature range from 20 to 300 °C is equal to 30 %, whereas that for  $\beta$ -CD makes up only 15 %. So, thermal destruction of *p*-ABA takes place also in this temperature interval. Decomposition of encapsulated *p*-ABA proceeds at higher temperature than that for individual compound. Hence, thermal stability of *p*-ABA increases. This phenomenon is caused by *p*-ABA molecule stabilization in the inner cavity of  $\beta$ -CD due to the nonspecific and specific intermolecular interactions. Thus, destruction of inclusion complex begins with the release of water molecules from the cavity of  $\beta$ -CD. Thereafter destruction of *p*-ABA and  $\beta$ -CD takes place. Thermal stability of  $\beta$ -CD in the inclusion complex does not differ from that of individual  $\beta$ -cyclodextrin because it occurs after decomposition of *p*-ABA.

It is known that  $\beta$ -CD crystallizes from aqueous solutions as either its undecahydrate or dodecahydrate, in which water molecules are included into hydrophobic

cavity of  $\beta$ -CD and also located in interstices between macromolecules. In aqueous solutions of  $\beta$ -CD energetically unfavoured water molecules can be readily substituted by appropriate less polar “guest” molecules, resulting in formation of inclusion complexes. Therefore the release of “high-enthalpy” water from the cavities of  $\beta$ -cyclodextrin to bulk water belongs to the one of the main driving forces for inclusion of “guest” molecules [12]. The repulsive forces between the incorporated water molecules and nonpolar  $\beta$ -CD cavity, on the one hand, and between bulk water and nonpolar “quest”, on the other hand, are two components of incoming process.

The content of water molecules in  $\beta$ -CD before and after complex formation with *p*-ABA was calculated on mass loss caused by release of water during thermogravimetric analysis (Figures 4, 5, TG curves). The activation energies of dehydration of  $\beta$ -CD and its inclusion compound with *p*-ABA were calculated by the Arrhenius relation:

$$E = \frac{RT_o^2}{b} \cdot \frac{1}{\tau}, \quad (1)$$

where  $E$  – the activation energy of dehydration,  $\text{J}\cdot\text{mol}^{-1}$ ;  $R$  – the universal gas constant,  $8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ ;  $T_o$  – the temperature of dehydration beginning, K;  $b$  – the heating rate,  $\text{K}\cdot\text{min}^{-1}$ ;  $\tau$  – the time is taken for attainment of maximum rate of dehydration, min.

Dehydration of  $\beta$ -CD (Fig. 4, DTG curve) occurs through a temperature range from 60 to 120 °C with maximum at 98°C. Total mass loss caused by dehydration of  $\beta$ -CD is equal to 12,6 % (Fig. 4) that corresponds to 7.9 water molecules localized in the cavity of  $\beta$ -CD (Table 1). Total mass loss calculated from the TG curve of “ $\beta$ -CD – *p*-ABA” inclusion complex in the temperature interval from 55 to 105 °C is lower than that for  $\beta$ -CD. It equals 4,2 % (Fig. 5) and corresponds to 2,6 water molecules which localized in the cavity of  $\beta$ -CD (Table 1). This is because the water molecules escape from hydrophobic cavity of  $\beta$ -CD as a result of “guest” compound incorporation.

## Conclusions

IR spectroscopy, X-ray diffraction and thermal analysis were used for characterization of the solid

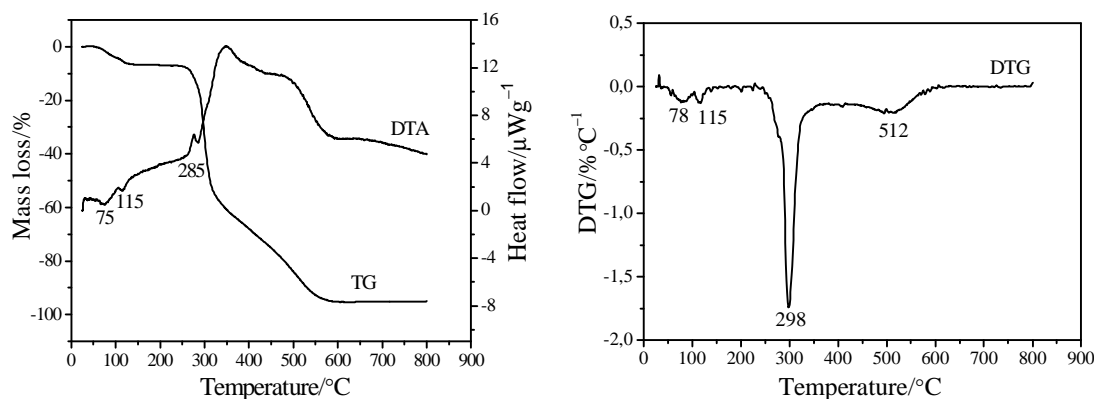


Fig. 5 Thermal analysis of „β - CD – p - ABA” inclusion complex

Table 1

Content of water and activation energies of dehydration for β-CD and “β-CD – p-ABA” inclusion complex

Compound	Content of water in β – CD			Activation energy of dehydration, kJ·mol <sup>-1</sup>
	mg/g β-CD	mmol/g β-CD	molecule/molecule β-CD	
β - CD	126	7,0	7,9	24,26
„β - CD – p - ABA”	42	2,3	2,6	44,72

“β-CD – p-ABA” supramolecular complex which was synthesized by coprecipitation from binary solution with stoichiometric ratio of components 1:1. The valence vibrations of the C=O bond in the carboxyl group of p - ABA, the deformation vibrations of the N–H bonds in the amino group, the valence vibrations of the C=C bonds in the benzene ring, and broadening of the absorption bands of the valence vibrations of the C–O bonds in the ether and hydroxyl groups of β - CD were

observed in the IR spectrum of “β - CD – p - ABA” inclusion complex. The reaching of amorphous phase as a result of „guest” molecule incorporation into the cavity of the „host” compound was detected. p - ABA in the inclusion complex exhibits high thermal stability due to its stabilization in the cavity of β - CD. It was found the enhancement of activation energy of dehydration and decrease of water content in the inclusion complex in comparison with β - CD. .

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**Вивчення твердого комплексу включення  
“ $\beta$ -циклодекстрин - пара-амінобензойна кислота” методами  
ІЧ спектроскопії, рентгенівської дифракції та термічного аналізу**

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В результаті співставлення даних ІЧ спектроскопії, рентгенівської дифракції та термічного аналізу (ДТА, ТГ, ДТГ) підтверджено взаємодію між компонентами твердого комплексу включення “ $\beta$ -циклодекстрин – пара-амінобензойна кислота”. Встановлено, що коливальні та обертальні рухи молекули “гостя” обмежено в результаті капсулювання пара-амінобензойної кислоти в порожнині  $\beta$ -циклодекстрину. Ступінь кристалічності супрамолекулярного комплексу суттєво зменшується порівняно з  $\beta$ -циклодекстрином та пара-амінобензойною кислотою. Спостерігається підвищення термічної стабільності пара-амінобензойної кислоти, що утворює комплекс включення з  $\beta$ -циклодекстрином. Було виявлено зменшення вмісту води у внутрішній порожнині  $\beta$ -циклодекстрину і збільшення енергії активації дегідратації в результаті утворення супрамолекулярної сполуки.

**Ключові слова:**  $\beta$ -циклодекстрин, пара-амінобензойна кислота, ІЧ спектроскопія, термічний аналіз, рентгенівська дифракція.