Journal of Molecular Structure 987 (2011) 225–231

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Cyclodextrin-based drug stabilizing system

N.V. Roik *, L.A. Belyakova

Chuiko Institute of Surface Chemistry of National Academy of Sciences of Ukraine, 17 General Naumov Str., Kyiv 03164, Ukraine

ARTICLE INFO

Article history:
Received 20 October 2010
Received in revised form 9 December 2010
Accepted 10 December 2010
Available online 21 December 2010

Keywords:
β-Cyclodextrin
Para-aminosalicylic acid
Thermodynamics
UV spectroscopy
IR spectroscopy
X-ray diffraction

ABSTRACT

UV spectroscopy study of *para*-aminosalicylic acid behaviour in the presence of β-cyclodextrin and without it in buffer solutions was realized. Influence of duration of contact, acidity of solution, temperature, and content of reagents in the binary solutions on the complex formation between β-cyclodextrin and *para*-aminosalicylic acid was examined. The stability constants of the supramolecular complexes formed at pH = 1.00 and pH = 6.86 were calculated by the Ketelar equation at various temperatures. It was found that the inclusion interaction of β-cyclodextrin with protonated type of *para*-aminosalicylic acid is superior to that for its anionic one. From the temperature dependence of stability constants the thermodynamic parameters involved in the complex formation (ΔG, ΔH, ΔS) were calculated. It was proved that complex formation between β-cyclodextrin and *para*-aminosalicylic acid is spontaneous process accompanied by the release of heat and decrease of entropy. To characterize the solid product of *para*-aminosalicylic acid inclusion into the cavity of β-cyclodextrin supramolecular complex with a 1:1 mole ratio of components have been prepared by kneading method and studied by IR spectroscopy and X-ray diffraction.

1. Introduction

para-Aminosalicylic acid (*p*-ASA) is structural analog of *para*-aminobenzoic acid possessing bacteriostatic activity [1]. It is of frequent use in combination with other anti-tuberculosis drugs for the treatment of various types of active tuberculosis, especially multi-drug resistant one [2–4]. The mechanism of *p*-ASA action is based on its ability to inhibit folic acid synthesis and prevent the multiplying of bacteria without destroying them [1]. However *p*-ASA is unstable substance and prompt production of a toxic inactive metabolite, *meta*-aminophenol, formed by decarboxylation of *p*-ASA under acid conditions causes complexities at its therapeutic application. The most commonly employed method to improve physicochemical properties of drug molecules is the preparation of its inclusion complexes with cyclodextrins (CD).

Cyclodextrins (CDs) are water soluble macrocyclic oligosaccharides with α-D-glucose units linked by α-(1 → 4) glycosidic bonds. Naturally occurring α-, β-, γ-cyclodextrins have the shape of a shallow truncated cone with the axial cavity lined with hydrogen atoms and glycosidic oxygen bridges. Consequently, the outer surface of this truncated cone is hydrophilic and the axial cavity is hydrophobic. The cavity of the CDs is occupied by included water molecules both in crystalline state as well as in aqueous solution. These included water molecules can be readily substituted by appropriate "guest" molecules which are less polar than water and fit geometrically into the CD cavity. Therefore CDs are able to form inclusion complexes with a wide variety of compounds ranging from very polar inorganic ions to completely nonpolar organic molecules. Binding specificity depends mostly on the size and geometry of "guest" compound. It is driven by noncovalent interactions such as van der Waals forces, hydrogen bonding and hydrophobic interactions [5,6]. Complex formation of CDs with "guest" compounds causes new physicochemical properties of the former. This is widely used to improve the solubility, bioavailability and stability of various drugs or biologically active compounds.

So, taking into account the ability of β-CD to change the physicochemical properties of "guest" compounds, it is very interesting to investigate the influence of added β-CD on the stability of *p*-ASA in the solutions. Therefore, in this paper the affect of contact duration, acidity of solution, temperature and content of reagents in the binary solutions on the complex formation between β-CD and *p*-ASA was studied by use of UV spectroscopy. Solid inclusion complex with a 1:1 mole ratio of components was obtained by kneading method. It was investigated by IR spectroscopy and X-ray diffraction analysis.

2. Experimental

2.1. Materials and chemicals

β-CD (from Fluka, purity ⩾ 99%), *p*-ASA (from ABCR, purity ⩾ 99%), standard volumetric solutions of phosphate buffer

* Corresponding author. Tel.: +380 44 422 96 91; fax: +380 44 424 35 67.
E-mail address: roik_nadya@ukr.net (N.V. Roik).

0022-2860/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molstruc.2010.12.027

and hydrochloric acid (all from RIAP, Ukraine) were used without additional purification.

2.2. Methods and instruments

UV spectra of *p*-ASA solutions were recorded in the 200–400 nm spectral range with a Specord M-40. Quarts cells with 2 and 1 mm path length were used. All spectroscopic measurements were made with temperature-controlled cell holder and water bath.

pH of *p*-ASA buffer solutions were measured by an Ionmeter I-120.1.

IR spectra of β-CD, *p*-ASA, their physical mixture and inclusion complex "β-CD–*p*-ASA" were recorded in the frequency range from 4000 to 1200 cm^{-1} with a Thermo Nicollet NEXUS FT-IR spectrophotometer using KBr pelleting.

X-ray diffraction spectra of *p*-ASA, β-CD, their physical mixture and "β-CD-*p*-ASA" inclusion complex were obtained by use of a diffractometer DRON-4-02 equipped with nickel filter. Samples were irradiated with monochromatised CuKα radiation (λ = 1.54178 Å) and registered in the angles range from 5 to 60 2Θ.

2.3. Procedure of kinetic experiments

Kinetic study of "β-CD–*p*-ASA" inclusion complex formation was carried out in phosphate buffer and hydrochloric acid solutions with pH = 6.86 and pH = 1.00, respectively. 10 ml aliquot of *p*-ASA (1.0×10^{-3} mol l^{-1}) solution with pH = 6.86 and 2.5 ml aliquot of *p*-ASA (2.0×10^{-3} mol l^{-1}) solution with pH = 1.00 were placed into a 50 ml volumetric flasks. Then 0–40 ml and 0–47.5 ml aliquots of β-CD solution (1.0×10^{-2} mol l^{-1}) and also appropriate amount of phosphate buffer and hydrochloric acid solution, respectively, were added up to constant volume (50 ml). The obtained solutions were shaken thoroughly for 24 h at room temperature.

The solutions were prepared just before taking measurements. Twice-distilled water was used for preparation of aqueous solutions. UV spectra were measured against reference solutions with the same reagent concentrations, but no *p*-ASA.

2.4. Procedure of complex formation study

Complex formation between *p*-ASA and β-CD was studied in phosphate buffer solutions with pH = 6.86 and hydrochloric acid solutions with pH = 1.00. In the case of phosphate buffer aliquots (on 1 ml) of *p*-ASA (1.0×10^{-3} mol l^{-1}) solution were placed into a 5 ml volumetric flasks and an appropriate amounts of β-CD solution (1.0×10^{-2} mol l^{-1}) prepared on a buffer solution with pH = 6.86 were added. When hydrochloric acid solution was used aliquots (on 0.5 ml) of *p*-ASA (1.0×10^{-3} mol l^{-1}) solution were placed into a 5 ml volumetric flasks and an appropriate amounts of β-CD solution (1.0×10^{-2} mol l^{-1}) prepared on a solution with pH = 1.00 were added. The obtained binary solutions were diluted to final volume with corresponding standard volumetric solutions of phosphate buffer and hydrochloric acid, shaken thoroughly, following equilibrated for 0.5 h in the 288–323 K temperature range.

2.5. Preparation of physical mixture

A physical mixture consisting of *p*-ASA and β-CD was prepared by admixing together *p*-ASA and β-CD in a 1:1 mole ratio in a mortar. It was pestle for 5 min to obtain a homogeneous blend.

2.6. Preparation of solid inclusion complex

The inclusion complex of *p*-ASA with β-CD was prepared by kneading method [7,8]. A physical mixture of components taken in a 1:1 mole ratio was wetted in a little twice-distilled water, mixed and kneaded thoroughly with a pestle in an agate mortar during 3 h. After drying in air at room temperature it was dried in an oven at 333 K for 2 h and stored in a desiccator.

3. Results and discussion

3.1. Kinetic study of "β-CD–*p*-ASA" inclusion complex formation

Decarboxylation of *p*-ASA in aqueous solutions makes difficult the quantitative determination of its content. The increasing amounts of the degradation product, *meta*-aminophenol, may cause significant decrease in optical density of *p*-ASA solutions at λ = 300 nm because of *meta*-aminophenol transparency at this wavelength [9,10]. Therefore study of the influence of duration of interaction between β-CD and *p*-ASA on the change in absorbance intensity at 300 nm was performed before determination of the stoichiometry of "β-CD–*p*-ASA" inclusion complex and calculation of thermodynamic parameters of its formation.

The effect of added β-CD on a stability of *p*-ASA in solutions with pH = 6.86 and pH = 1.00 is shown in Fig. 1. It can be seen that the degradation of *p*-ASA in buffer solution with pH = 6.86 is negligible (Fig. 1a). Addition of β-CD causes a slight shift of the absorption maxima of *p*-ASA registered at 209–301 nm and decrease of its absorption intensity, attributable to the formation of inclusion complex between β-CD and *p*-ASA. The decrease of absorbance may be caused by the influence of high electron density inside the β-CD cavity on the "guest" molecule and partial shielding of the chromophore electrons [11,12]. Decarboxylation of *p*-ASA in acidic medium proceeds more rapidly, but during the first 5 h no visible changes in the absorbance of *p*-ASA are observed (Fig. 1b). The change of absorption intensity of *p*-ASA solutions (pH = 1.00) with the amount of added β-CD is contrary to that at pH = 6.86. Addition of β-CD causes shift of the absorption maxima of *p*-ASA from 301 nm to 303 nm and a noticeable increase of its absorption intensity. The registered bathochromic shift and increased intensity of absorption band indicate either the existence of hydrogen bonds between the "host" and the "guest" molecules or weak van der Waals forces involved in the complex formation process [13].

The relation between absorption intensity decreasing and duration of experiment indicates that the hydrolysis of *p*-ASA encapsulated in β-CD is slightly slower than that of initial *p*-ASA. It can be concluded that during the time required to attain equilibrium of complex formation (0.5 h) decarboxylation of *p*-ASA does not occur.

3.2. Stability constants of "β-CD–*p*-ASA" inclusion complexes

It is known that the inclusion interaction of β-CD with uncharged species of organic molecules is superior to that for its anionic and cationic types [14–17]. However the uncharged form of *p*-ASA coexists with an unstable zwitter-ion (Scheme 1) which is converted to *meta*-aminophenol at decarboxylizing [9]. In the work [18] the ionization constants for *p*-ASA, determined by the multiwavelength spectrophotometric titration method, are reported: pK_{a1} = 1.79, pK_{a2} = 3.63 (pK_{b1} = 12.21, pK_{b2} = 10.37). Taking into account aforesaid, we have investigated the inclusion complex formation of β-CD with anionic and cationic types of *p*-ASA in aqueous solutions at pH = 6.86 and pH = 1.00, respectively, where the content of zwitterionic form of *p*-ASA is negligible and degradation of *p*-ASA does not occur. The effect of β-CD concentration on the absorption intensity of *p*-ASA has been examined. The UV spectra of *p*-ASA in the presence of various concentrations of β-CD are shown in Fig. 2. There are three maxima for *p*-ASA