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Physicochemical characteristics of the inclusion complexes of biologically active compounds with 2-hydroxypropyl-β-cyclodextrin

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ABSTRACT

Inclusion complexation of 2-hydroxypropyl-β-cyclodextrin with biologically active compounds 2-hydroxybenzaldehyde isonicotinoylhydrazone and 2-hydroxy-3-methoxybenzaldehyde isonicotinoylhydrazone in buffer pH 7.4 solution and in solid state have been investigated. The solubility of compounds at different concentrations 2-hydroxypropyl-β-cyclodextrin was studied by phase solubility method. The profile displayed a typical A_L-type, indicating the formation of 1:1 stoichiometric inclusion complex. Solution state complexation in buffer was validated by ultra violet absorption. Stoichiometry of complex was confirmed by Job's method. The water solubility of compounds studied was significantly increased through complexation with 2-hydroxypropyl-β-cyclodextrin. Complexation stability constants and thermodynamic parameters have been calculated. It has been concluded that inclusion complexation is an exothermal and enthalpy-determined process. The solid state inclusion complex was prepared by the ball-milling method and characterized by differential scanning calorimetry, Fourier-transform infrared spectroscopy and X-ray powder diffraction. The dissolution rate of the active pharmaceutical ingredient of the supramolecular complexes were obtained.

1. Introduction

Compounds 2-hydroxybenzaldehyde isonicotinoylhydrazone (I) and 2-hydroxy-3-methoxybenzaldehyde isonicotinoylhydrazone (II) (Fig. 1A) are quite promising for developing drugs possessing a high antitubercular, antiasthmatic, broncholytic action that is not worse and sometimes is better than that of drugs currently used in medicine [1–4]. Biological tests have shown that these compounds are active on models of histamine-induced bronchospasm, sephadex-induced bronchoalveolitis, and mycobacterium tuberculosis strains. The main problem of creating dosage forms, parenteral and inhalation ones in particular, based on the above-mentioned compounds and their further use is the extremely low solubility of the latter in water solutions [5].

Cyclodextrins belonging to the regular cyclic oligosaccharides consisting of 6 (α), 7 (β) and 8 (γ) glycopyranozic units connected by α-(1,4) bonds, have been recently widely used as additive agents in biochemistry and pharmacology where they are primarily applied to encapsulation of different pharmaceutical media. Such encapsulation usually protects a drug from biodegradation, increases its solubility in water, and in a number of cases makes drug delivery to the target organ more effective and selective [6–9]. Native CDs are known to interact with human tissues and to extract cholesterol and other cell membrane components, particularly if renal tubules are accumulated in the cells, thus having a nephrotoxic effect [10]. It should be noted that β-cyclodextrin (β-CD) possessing the most pronounced complexation ability is rather poorly soluble in water and organic solvents, which limits its use in pharmaceutical compounds [7].

Modified cyclodextrin—2-hydroxypropyl-β-cyclodextrin (2HP-β-CD, Fig. 1B) includes seven D-glucopyranoside fragments containing hydroxypropyl groups in the sixth position. A hydrophobic cavity and good solubility in water provided by hydrophilic alcohol groups gives 2HP-β-CD a unique ability of complexation with molecules of different organic substances of respective sizes and polarity. 2HP-β-CD is widely used for drug encapsulation due to its inclusion ability, good solubility in water and low toxicity [10–13].

The aim of this work is to establish a possibility of obtaining inclusion complexes of biologically active compounds with

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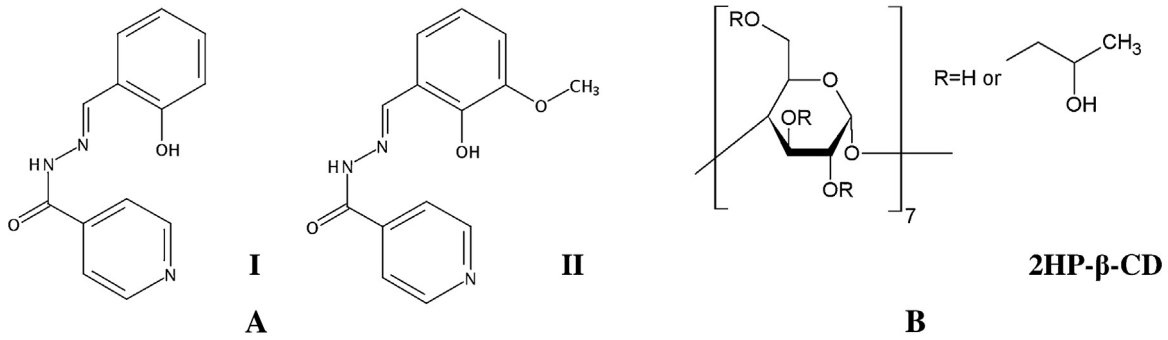

Fig. 1. Chemical structure of compounds studied (A) and 2HP-β-CD (B).

2-hydroxypropyl-β-cyclodextrin and improving its solubility in pharmaceutically relevant buffer pH 7.4. The inclusion complexes were prepared in solid state and systematically characterized by phase solubility diagram, UV–vis spectroscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray powder diffraction. This study is directed to provide an efficient approach to developing new biologically active compounds with improved water solubility and low toxicity.

2. Materials and methods

2.1. Materials

2-hydroxybenzaldehyde isonicotinoylhydrazone (I) and 2-hydroxy-3-methoxybenzalde-hyde isonicotinoylhydrazone (II) were synthesized by the methods described in literature [2,3]. X-ray structure determinations revealed that the molecules of both compounds display *trans* configurations with respect to the C=N double bonds [14]. The structure of the obtained compounds were confirmed by Fourier-transform infrared spectroscopy and element analysis. Compound I:Anal. Calc. for $C_{13}H_{11}N_3O_2$: C 64.73%, H 4.60%, N 17.43%. Found: C 64.90%, H 4.64%, N 17.60%. FT-IR (KBr, cm^{-1}): ν = 3170, 1670, 1600, 1560, 1280, 1150, 770. MS: *m/z* 241. Compound II Anal. Calc. for $C_{14}H_{13}N_3O_3$: C 61.99%, H 4.83%, N 15.50%. Found: C 62.17%, H 4.70%, N 15.55%. FT-IR (KBr, cm^{-1}): ν = 3200, 1680, 1600, 1560, 1280, 1245, 970, 735. MS: *m/z* 271. 2HP-β-CD with substitution degree 0.6 was received from Aldrich.

Purity of the samples was tested using thin layer chromatography (TLC) aluminum plates with macroporous gel "Silufol UV-254" (Czech Republic) size 20 × 20 cm. The mixture of acetone:ammonium:hydroxide:ethanol/9.8:0.4:0.25 (v/v/v) was used as an eluent. Chromatography was carried upward method. Detection zones substances chromatograms performed under ultraviolet light at 254 nm. The value of ratio of fronts (R_f) was 0.24. The compounds was purified by recrystallization with absolute ethanol. The purity of compounds after purification was determined by HPLC an Agilent 1100 series apparatus with a Kinetex C18, 2.6 μm, 3 × 100 mm (Phenomenex, USA). The mobile phase consisted of a mixture of water-methanol (42/58, v/v). The flow-rate was 0.4 ml/min. The detector was operated at 294 and 308 nm, respectively. The injection volume was 10 μl. A. The major information of the used chemicals is given in Table 1.

As the water phase we used a phosphate buffer modeling medium of the blood plasma. Bidistilled water (with electrical conductivity 2.1 $\mu S\,cm^{-1}$) was used for preparation of buffer solutions. Phosphate buffer (0.067 M, pH 7.4) was prepared combining the KH_2PO_4 (9.073 g in 1 l H_2O) and $Na_2HPO_4{\cdot}12H_2O$ (23.611 g in 1 l H_2O) salts. Ionic strength I = 0.15 mol/l was adjusted by adding potassium chloride (1.291 g in 1 l H_2O) [15]. Compounds were weighed in AND Gemini Analytical Balance GR-202 (Japan). Standard uncertainity of measurement mass of samples was *u(m)* = 0.01 mg. The solutions pH was measured by using pH meter FG2-Kit (Mettler Toledo, Switzerland) standardized with pH 1.68, 6.86 and 9.22 solutions.

2.2. Preparation of inclusion complex in solution and solubility measurements

Phase solubility studies were performed according to the method reported by Higuchi and Connors [16]. Excess amount of the compounds studied was added to 20 ml of phosphate buffer solution pH 7.4 containing various concentrations of 2HP-β-CD (0–0.09 mol kg^{-1}). The prepared solutions were placed into glass test tubes and the suspensions were shaken on rotary shaker at 298.15, 303.15, 308.15, 313.15, 317.15 ± 0.05 K for 72 h in the thermostatically box. Saturation was confirmed by the presence of the undissolved part of substance. Once the equilibrium was achieved, the saturated solution was taken and centrifuged (Biofuge stratus, Germany) under the temperature control for 5 min at a fixed temperature. The solid phase was removed through isothermal filtration by the filter MILLEX®HA 0.20 μm (Ireland). An aliquot of the saturated solution was taken at fixed temperature using the thermostated equipment and then diluted by the solvent. The absorbance was measured spectrophotometrically at room temperature. The experimental results are reported as an average value of at least three replicated experiments.

The calibration procedure was made at room temperature using the solutions with known concentrations of each substance in buffer pH 7.4 and buffer pH 7.4 with different 2HP-β-CD concentrations. The solutions were prepared by adding an appropriate mass of substance and buffer pH 7.4 to the flask and mixing until the substance was totally dissolved. The absorbance of the solutions was measured and calibration curves were constructed.

The complexes stoichiometry was measured by the isomolar series method (Job's method) [17]. Equimolar solutions of compounds I ($7.54\,10^{-5}$ mol kg^{-1}), II ($1.03\,10^{-4}$ mol kg^{-1}) and 2HP-β-CD were mixed to a standard volume (1 ml: 9 ml; 2 ml: 8 ml; 3 ml: 7 ml and so on) varying the molar ratio but keeping the total concentration of the species constant. After stirring, the absorbance (D) at λ_{max} was measured for all solutions and the difference in absorbance (ΔD) in the presence and in absence of 2HP-β-CD was plotted against R (R = [Drug]/[Drug] + [2HP-β-CD]).

2.3. Preparation of the physical mixtures and inclusion complexes in solid state

Physical mixtures compounds studied with 2HP-β-CD (1:1 molar ratio) were prepared by gentle mixing of the accurately weighed components in a mortar with a spatula.

Complexes compounds I, II with 2HP-β-CD of 1:1 M ratios were prepared by ball-milling method using a planetary micro mill Pul-