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Supramolecular Complexes of β-Cyclodextrin with Conjugated Fatty Acids: Synthesis and Stability

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***Abstract*—The supramolecular complex formation in aqueous solution between fatty acids (with the content of α-eleostearic acid more than 70%) and β-cyclodextrin, βCD, was supported by FT-IR and UV spectroscopies as well as by XRD-analysis. RP HPLC was utilized for investigation of the content of fatty acid in the inclusion complex and their stability. All results showed that the molecules of fatty acids were successfully included in the β-cyclodextrin cavity. The yield of the inclusion complex is up to 30.1% with a fatty acid content of 20.5%. Due to the formation of inclusion complex, the solubility, and stability of conjugated acids significant increase.**

Keywords—inclusion complexes, β-cyclodextrin, α-eleostearic acid, yield, composition

I. INTRODUCTION

The conjugated linolenic acids (CLnA), are positional and geometric isomers of octadecatrienoic acids with three conjugated C=C-bonds, recently have been given special attention in pharmacology and the paint industry [1, 2]. CLnAs were demonstrated to have high biological activity, including anti-carcinogenic and antigenic effects, strengthening the immune system and anti-diabetic action [3, 4]. The bioaccessability of CLnAs as others lipophilic compounds is limited because of low water solubility and oxidative instability of conjugated polyunsaturated compounds. The instability of CLnA was estimated in paper [5], where the octadecatrienoic acid isomers were completely destructed by stirring in the air for 30 min at 50ºC. The development of modern technologies using formation of supramolecular guest-host complexes is of a great importance for solving these problems. Beta-cyclodextrin (βCD), both native and modified, consisting of seven glucose units, represent a unique compound capable for the formation of inclusion complexes with various lipophilic organic substances by molecular encapsulation or a hostess-guest complex [6]. According to the literature data [7, 8], lipophilic parts of guest molecule can be included in the lipophilic cavity of β-cyclodextrin (βCD) which could improve the water solubility of lipophilic molecule. In the present work, we developed a method for producing supramolecular complexes of α-eleostearic acid with βCD and investigated the stability of conjugated acids in the obtained complexes.

II. EXPERIMENTAL

Preparation of inclusion complex (IC) of fatty acids with βCD preparation

The fatty acids (FA) were obtained by saponification of Momordica cochinchinensis seed oils with 2M solution of NaOH in ethanol. The saponification was performed at a room temperature for at least 2.5 hours. After saponification, the mixture was treated with concentrated HCl solution until obtaining the solution with pH ≈ 3. The prepared fatty acids were extracted by *n*-hexane. The *n*-hexane solution was evaporated under vacuum in a rotary evaporator and FAs were dissolved in ethanol for the following analysis.

The inclusion complex was prepared by homogenization of aqueous solutions of βCD (10 mM) with a different volume of solution FAs in ethanol (60 mg/ml) using a homogenizer (US-4102 Ulab - 25000 gm). The mixture was stirred lightly for 2 hours for the equilibration of the complexation. Inclusion complexes form a white precipitate. The precipitate was separated by centrifugation, washed with 10 ml of water three times and freeze dried (Freezone 2.5 Labconco at 0.021 mbar and -48 ° C).

The content of fatty acids in the complex was determined by RP-HPLC using a calibration curve plotted for sample acid solution with concentrations 0.03-10 mg/ml. To the 20 mg of the complex 3 ml of acetone was added, the mixture was stirred for 15 minutes, then the β-cyclodextrin was

separated by centrifugation and supernatant was collected for fatty acids determination by RP HPLC. The process was repeated three times for exhausting extraction of FAs from the inclusion complex. The extraction completeness was controlled by comparing the IR spectra of βCD and the powder after extraction. A yield of inclusion complex (dry weight powder) was calculated by the equation:

$$Yield = \frac{m_{dry\_powder}}{m_{FA} + m_{bCD}} 100(\%)$$

Analysis of inclusion complex

FT-IR spectra were recorded using a spectrometer Shimadzu IR Prestige for samples in KBr disks. The scans were acquired at a resolution of 4.0 cm^{-1} (from 450 to 4000 cm^{-1}).

The diffraction patterns of βCD, and of inclusion complexes were registered using an X-ray diffractometer Rigaku Ultima IV using detector DTEX / ULTRAT. CuKα radiation (λ = 1,5406 Å) applied to measure the diffraction angles in the region 5° to 50° under a voltage of 40 kV and current of 30 mA and at a speed of 5°/minute. The diffraction pattern of βCD was compared with standard diffraction from the library via Jade 5 software to recognize crystalline structures.

To study the complexation product by spectrophotometric method, 10 ml of water was added to a small portion of the complex and the mixture was shaken for 6 hours at 25°C to obtain a solution of the complex in water. The solution was separated from the insoluble part of the complex by filtration through a 0.45 μm PTFE HP filter, and the electronic absorption spectra of the resulting solutions were recorded in quartz cuvettes by UV-2550 Shimadzu spectrometer.

Stability test

Stability test of inclusion complexes (after drying) and that of physical mixture (PM) with the same proportion of FAs and β-CD used as the control mixture were investigated. The samples were stored in the refrigerator. After a specified time, 20 mg of each samples were withdrawn and analyzed by RP-HPLC method.

Chromatograph LC20 (Shimadzu) was used with column: 4.6×250 mm Kromasil 100-5C18, column thermostat temperature 30ºC, mobile phase: acetone: acetonitrile: acid acetic (20:80:1 by volumes), flow rate: 0.8 ml/min: refractive index detector RID 10A.

III. RESULTS AND DISCUSSION

Synthesis and feature of supramolecular complex

The complex of FAs with β-CD was prepared by addition of different amounts of FAs ethanol solutions to 20 ml of 10 mM β-CD solution in water. The results of complexion yield determination and the content of fatty acids in the complex were presented in Fig.1.

The optimal ratios of fatty acids and β-CD (in the form of a 10 mM aqueous solution) have been established, which make it possible to achieve an inclusion complex yield of up to 30.1 % with a fatty acid content of 20.5% in the complex.

The obtained inclusion complexes were studied by IR spectroscopy, X-ray phase analysis, and UV spectroscopy and HPLC methods after their destruction with acetone.

It has been found that the formation of a complex changes the initial crystal lattice of β-CD and, therefore, an X-ray diffraction pattern. That for initial βCD indicates high crystallinity, due to characteristic sharp peaks (Fig. 2A), that is in coincidence with published data [9]. The diffraction pattern of βCD was dissimilar to the spectrum of the inclusion complex indicating less ordered structure with the peaks appeared at 6.52°, 11.52°, 17.42°, 17.94º, 21.36º and 23.76° (as shown in Fig. 2b). Since the obtained complex showed new reflectance peaks, the formation of inclusion complex between FAs and β-CD was proved.

Moreover, in the infrared spectrum of the complex (B), all characteristic βCD bands were preserved and FAs bands were added, Fig.4.

Due to the formation of the complex, an increase in the intensity of the absorption band is observed in the region of 2800–3000 cm^{-1}, which is characteristic for (sp^3)C-H stretching vibrations. The stretching vibration of C=O group is shifted to 1689.6 cm^{-1} and its intensity decreases (compared to the spectrum of fatty acids).

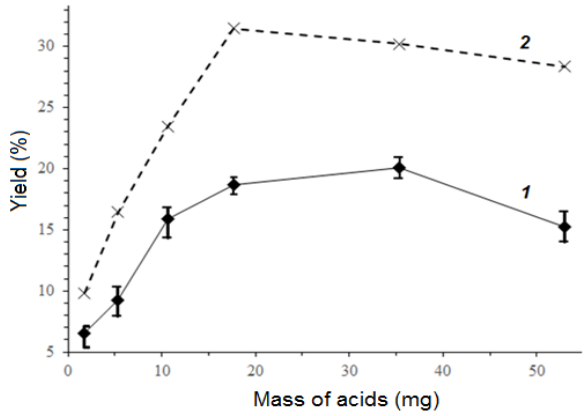

Fig.1. The dependence of the yield of inclusion complexes (2) and mass fraction of fatty acids in them (1) on the mass of added FAs.

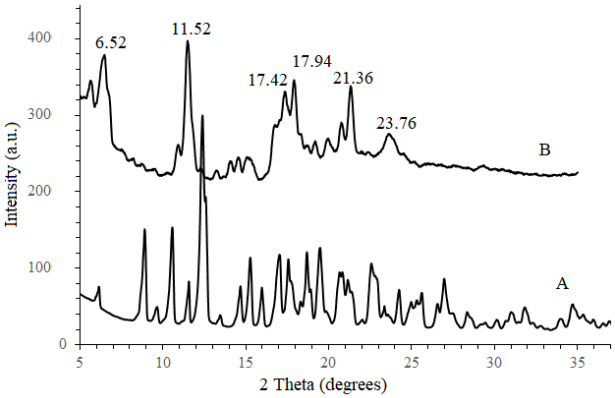

Fig.2. Diffraction patterns of β-CD (A), Fatty acid-βCD complex (B).

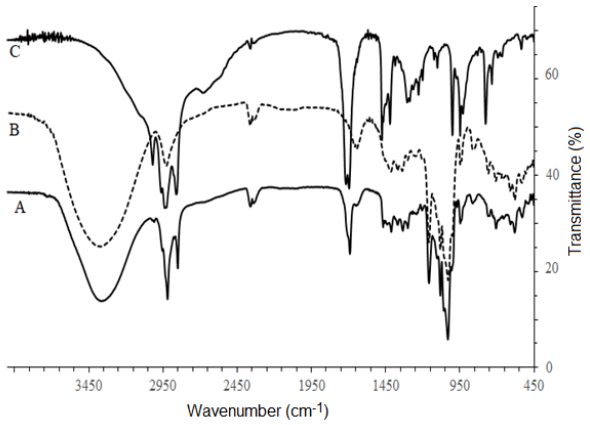

Fig.3. IR spectra FA-βCD inclusion complex (A), βCD (B), fatty acids (C).

The solubility and feature UV spectra of supramolecular complex were studied and showed in Fig. 4.

As following from the presented results, the UV spectrum of the αE/βCD inclusion complex is noticeably different from the spectrum of α-eleostearic acid in ethanol and from the spectrum of saturated suspension of acids in water after stirring. The initial spectrum has an electronic-vibrational structure being characteristic for conjugated polyenes (with absorption maxima at 282, 271 and 263 nm), which disappears in water solutions and two new broad bands with hypsochromic and bathochromic shifts (232 and 283 nm) appear. An increase in the absorption intensity compared with a spectrum of acid suspension in water without cyclodextrin addition indicates the expected increase of acid solubility in water, i.e. - on the formation of inclusion complexes. Finally, the formation of two new bands indicates the association of α-eleostearic molecules forming H-aggregates (with a hypsochrome shift) and J-aggregates (with a bathochromic shift). According to the molecular exciton model [10], the H-association of chromophores of α-eleostearic acid (the chromophores are parallel to each other with a slight shift or without a shift) results in a hypsochrome shift of the absorption band. Meanwhile the J-association (alignment of the chromophores in the type "head to tail") results in the bathochromic shift of the absorption bands.

To assess the feasibility of implementing various association options in inclusion complexes, in this work, computer simulation was performed when geometry was optimized using the MM+ method in the HyperChem 6.0 software environment, Fig. 5.

The initial modes of the mutual orientation of the molecules used to calculate the geometry of the structures were created by the Chem3D program and then imported into HyperChem for structure optimization.

According to modeling, it was found that even three molecules of fatty acid might enter one cavity of cyclodextrin. If two acid molecules and one βCD molecule are involved the H associates are formed, which is in good agreement with the appearance of a broad band with a hypsochrome shift. Therefore, to model J-associates, it is necessary to construct more complex structures with optimization of geometry directly in several cavities of cyclodextrins.

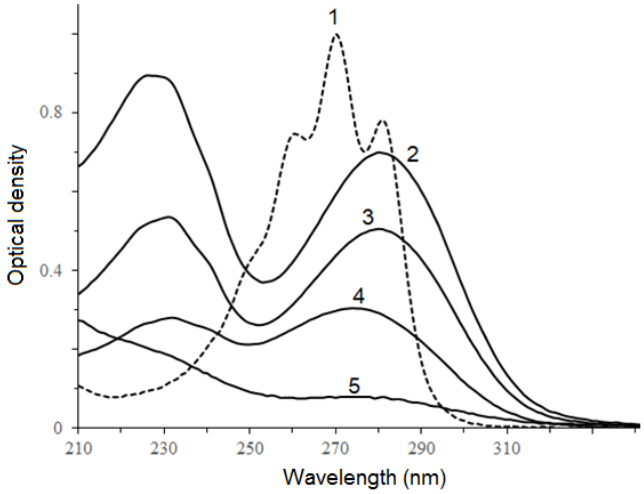

Fig. 4. UV absorption spectrum of α-eleostearic acid in: 1- ethanol, 2 - 12 mM, 3 - 10 mM, 4 - 6 mM, 5 - 0 mM of βCD concentration in water.

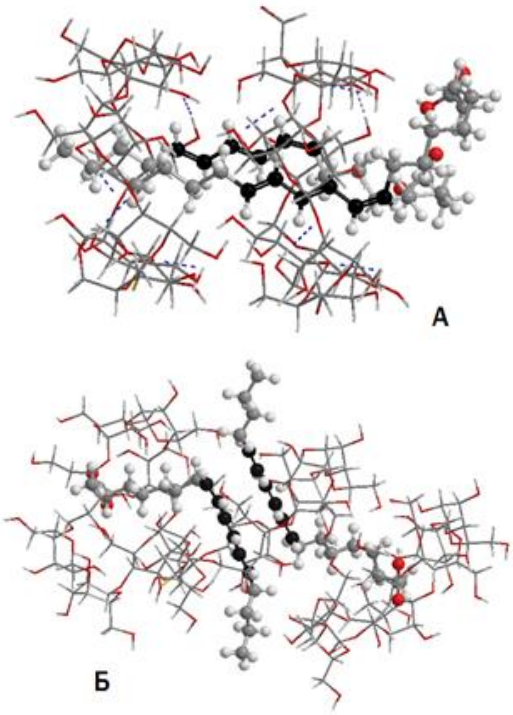

Fig. 5. Geometric structures of supramolecular complexes αE/βCD with a different ratio: A - 2: 2, B - 2: 3.

Stability of α-eleostearic acid in inclusion complex

The stability of conjugated fatty acid in complexing state was investigated and the results were shown in Fig. 6.

According to presented data, the entry of α-eleostearic acid into the cyclodextrin cavity in deed increases stability (less than 5% of the amount of conjugated acid disappears after 96 hours after drying) while for the physical mix the conjugated acid was oxidized about 90%.

However, the result showed that α-eleostearic acid disappears almost completely after 250 hours of storage. This instability of conjugated fatty acid in cyclodextrin's cavity

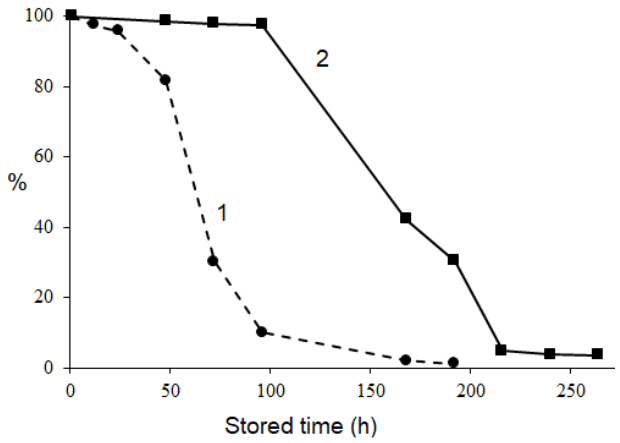

Fig. 6. Stability of α-eleostearic in the physical mixture (1) and in inclusion complexes (2)

seems to be related to their association that is favorable for polymerization in the presence of carboxylic groups. This was confirmed the by significant growth of stability due to transformation of the acids into the calcium salts. According to our data, this results in only 10% loss of α-eleostearic acid even after 23 days storage in refrigerator.

IV. CONCLUSIONS

The supramolecular inclusion complexes of α-eleostearic acid with β-cyclodextrin were prepared as white precipitate from water solution of β-cyclodextrin after addition of ethanolic solution of acids. The formation of the complex has been confirmed by IR- and X-ray diffraction investigations. The optimized yield and composition of complex was estimated by HPLC. Complexation really increases solubility of α-eleostearic acid in water and stability during storage, though the latter might be significantly improved by neutralization of carboxylic group.

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