Characterization and Comparison of the Solid State Inclusion Compounds of α- , β- Cyclodextrins and its 2-Hydroxypropyl Derivatives with Uracil and 5-fluorouracil

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The objective of the present work was to obtain and evaluate the solid inclusion complexes of α-, β-cyclodextrins and hydroxypropyl-α-, hydroxypropyl-β-cyclodextrins derivatives with uracil (U) and anti-cancer agent 5-fluorouracil (5FU). The thermogravimetry (TG), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR) and scanning electron microscopy (SEM) were used to characterize these inclusion compounds. New information on the physicochemical behavior of these solid state complexes synthesized by melting in solution was obtained. Corelating the experimental data we found that the complexation occurs for each host:guest system thus, the preparation method exhibited a real efficiency to interaction achievements. The inclusion capability and the thermal stability of the studied complexes were due to both the flexibility and the size of the cyclodextrin ring as well the stereoelectronic effect of the U or 5FU molecule. We appreciated that the complexes between U or 5FU with 2-hydroxypropyl derivatives of α- and β- CD have the largest content of guest and are the most stable. The obtained results could be useful in formulation of the pharmaceutical products and also in drug delivery.

Keywords Cyclodextrin, Uracil, 5-Fluorouracil, Inclusion Complexes, Thermal Behavior

Cyclodextrins (CD) (cyclomaltooligosaccharides) are macrocyclic oligosugars capable to form inclusion complexes with small hydrophobic molecules, improving their properties. Cyclodextrins can be obtained from starch by enzymatic conversion. The enzyme modifies the polysaccharide, turning it into a cyclic oligomer with a proper number of glycopyranoside units. Cyclodextrins are shaped like an intrusive truncated cone which is a relatively hydrophobic in the middle and relatively hydrophilic on the outside because of hydroxyl groups. Thus, cyclodextrins are designed to have a high ability to complex a wide range of molecules with different degree of hydrophobicity. The most important factor in the guest selectivity is the size of the cyclodextrin cavity matching that of the guest molecule [1, 2].

When a guest molecule fit properly within the CD's cavity the resulting system can have highly different properties compared to that of the guest molecule alone. The molecular encapsulation phenomenon may occur both in solution and in solid state. In solid state, the guest molecule can be totally encapsulated or may perform a reduced possibility of accommodation within the CD's cavity [3]. Thus, cyclodextrins are the most studied host molecule in supramolecular chemistry because of their advantages as including increased solubility, stability, poor reactivity, volatility and bioavailability [1]. Uracil (U) and 5-fluorouracil (5FU) are pyrimidine derivative compounds with weak acidity behavior and depending upon the available *light [4].* The biochemical importance of U molecule is well known [4, 5]. U guest is a nucleic acid constituent used in organic synthesis and in *pharmaceutical* formulations. The presence of electron donating groups in its structure leads to derivatives series of cytotoxic compounds such as 5FU which is a substituted U that differ in structure by only a fluorine atom at C5. The dividing mechanism of cancerous cells is corrupted when synthesis of the pyrimidine thymidine is stopped therefore 5FU acts as a thymidylate synthase inhibitor when replace U during the nucleic acid replication process [5]. 5FU is used in synthesis of the 5FU prodrugs which are generally more toxic and hydrophobic than 5FU. The anti-tumoral capabilities of 5FU antimethabolite drug and its therapeutic formulation have been discussed in many papers [6, 7].

In the present work, U and 5FU were chosen as guest molecules in terms of possibility to compare the resulted inclusion compounds and to find in what manner fluorine atom attached at C5 position in uracil molecule influences the properties of obtained complexes. Further, the encapsulation of 5FU in CD's cavity comes to improve the drug properties in order to control its release from thermosensitive formulation and to avoid the chemical incompatibility in some medical treatments. Even though the characterization of cyclodextrin inclusion complexes with 5FU was accomplished in some papers, according to our knowledge, there is no a complete characterization of the CD/U and CD/5FU complexes. In order to obtain further insights into physical mechanisms explaining the formation of the solid state inclusion compounds between host molecules and guests U or 5FU, in our investigations we employed DSC, TG/DTG, FTIR and SEM techniques. The information provided in this paper may be useful to make clear the relationship between the thermodynamic stability and the structure of the compounds and also to prove the complexation efficiency.

Experimental part

Materials

The uracil (U) - purity 99% was purchased from Loba Chemie, 5-fluorouracil (5FU) - purity 99%, α-Cyclodextrin

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(αCD) - purity 98%, β-Cyclodextrin (βCD) - purity 97%, (2-Hydroxypropyl)-α-Cyclodextrin (HPαCD), (2-Hydroxypropyl)-β-Cyclodextrin (HPβCD) were purchased from Sigma Aldrich Chemical Company. All substances were used without further purification.

Preparation of solid state samples

The binary physical mixtures (p.m.) of uracil (U) and 5-fluorouracil (5FU) with each selected host were prepared at 1:1 molar guest:host ratio by grinding and homogenizing in normal lab conditions.

Preparation of inclusion complexes

The solid state inclusion compounds were prepared in 1:1 molar ratio of the host and guest by melting in solution method. The host molecule (each of αCD, βCD, HPαCD, HPβCD) was dissolved in double distilled water (4 mL) at 60°C, then the guest (U or 5FU) was added in solid state and the mixture was stirred (600 rpm) for 2h at 60°C. The mixture solution was cooled at room temperature. The white precipitate was filtered on a G4 glass filter and was washed with ethanol for purification and dried under vacuum at 40°C.

Apparatus and methods

Differential scanning calorimetry (DSC)

The DSC curves were recorded on a Perkin Elmer Diamond DSC under a heating rate of 10 K/min over a temperature range (0–400)°C. The apparatus was calibrated for temperature and enthalpy by melting high purity indium. The instrument was flushed with argon atmosphere (20 mL min^{-1}). Sample of 1–5 mg were transferred into aluminum crucibles which were sealed and weighed with the Partner XA balance, with a precision of 10 μg. DSC curves were recorded for pure compounds (CDs, uracil and 5-fluorouracil), physical binary mixture of CDs/U and CDs/5FU and for solids containing corresponding inclusion complexes.

Thermogravimetry (TG)

Thermogravimetric data of the inclusion compounds and pure uracil, 5-fluorouracil and cyclodextrins were obtained using a model Setaram Setsys Evolution 17 with open alumina crucibles of 100 μL volume. The calorimeter was calibrated using recommended standards of indium (ΔH_{fus} = 28.46 J g^{-1}). In all measurements the calorimeter was operated at a heating rate of 10°C/min in the temperature range 20 to 450°C. All samples masses have been between 1 and 2 mg and were scanned in flowing argon atmosphere (16 mL min^{-1}).

Fourier transform infrared spectroscopy (FTIR)

FT-IR spectral data of pure compounds and inclusion complexes were recorded at room temperature by Nicolet iS10 FT-IR Spectrometer covering the range of 4000 to 600 cm^{-1}. The spectra were acquired with an average of 32 scans with spectral resolution of 4 cm^{-1} in attenuated total reflectance (ATR) mode.

Scanning electron microscopy (SEM)

The morphology of the samples were investigated by scanning electron microscopy (SEM) using a high-resolution microscope, FEI Quanta 3D FEG model, operating at 15kV, in low vacuum mode with Low Vac Secondary Electron Detector (LVSED). Samples preparation was minimal and consisted in immobilizing the material on a double-sided carbon tape, without coating.

Results and discussions

The DSC curves presented in figures 1-4 were recorded for pure compounds (CDs, U and 5FU), physical binary mixture of CDs/U and CDs/5FU and for inclusion complexes. The thermal events for pure CDs were reported in many studies being related for many types of commercial CDs.

As can be seen in figures 1 and 2, the DSC curve of pure αCD shows a broad endothermic peak between 34 and 130 °C corresponding to its dehydration and reorganization and a thermal effect starting at 298 °C which is attributed to a melting - decomposition process of αCD, [8]. For βCD the loss of water emerged between 50 and 110 °C and the small peak at around the temperature of 240°C corresponds to an irreversible solid–solid phase transition which is finally followed by a degradation process starting at around the temperature of 290°C. This thermal behavior of βCD is consistent with literature, [9, 10]. The thermograms of each of the pure HPαCD and HPβCD, (figs. 3, 4), show a broad endothermic effect with a maximum temperature at around 60°C associated to dehydration of compound followed by thermal decomposition, at temperature of 295 °C for HPαCD and at temperature of 315 °C for HPβCD. The DSC curve of U showed two overlapped peaks corresponding to melting and decomposition respectively. The temperature of the melting obtained from U curve is 334.1°C and the corresponding melting enthalpy value is 97.39 J/g. The DSC thermogram shows two endothermic thermal events for the 5FU drug. The first event was attributed to the melting process of 5FU and starts at temperature of 280.9 °C and the second was the thermal decomposition and started at temperature of 333.4 °C. The registered melting event is characterized by enthalpy value of 223.71 J/g which is comparable with literature data [11].

In thermogram corresponding to inclusion complexes αCD/U and βCD/U (fig. 1), the peak attributed to melting process of uracil is not present. In the same time, the peak attributed to dehydration process of αCD is smaller, which sustain the replacing of water molecules due to encapsulation of U in αCD cavity. A possible blend of inclusion complex and free αCD can be taken into account as the peak corresponding to dehydration process is still present. A high thermal stability for αCD/U and for βCD/U is proved by the high value of starting temperature of the decomposition event. As one can observe in figure 1, the DSC curve of physical mixture αCD/U p.m. is comparatively similar with βCD/U complex curve, which suggest the formation of the inclusion complex, while for the βCD/U p.m. indicate a slight interaction between the components.

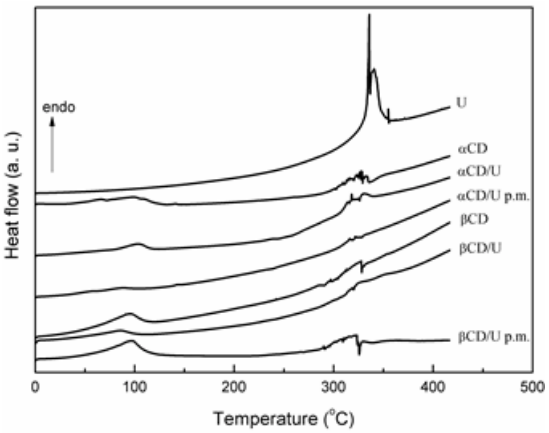

Fig. 1. DSC diagrams for uracil (U), cyclodextrins (αCD and βCD), inclusion complex (αCD/U and βCD/U) and physical mixture (αCD/U p.m. and βCD/U p.m.)

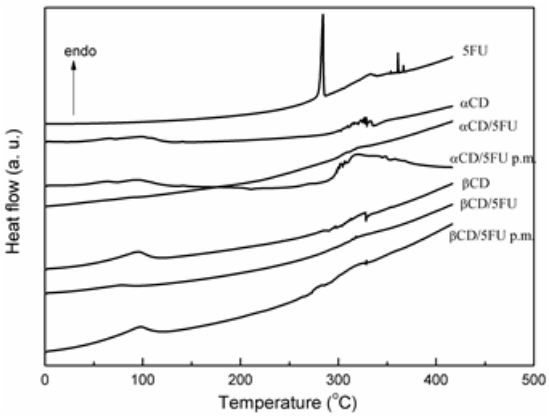

Fig. 2. DSC diagram for 5-fluorouracil (5FU), cyclodextrins (αCD and βCD), inclusion complex (αCD/5FU and βCD/5FU) and physical mixture (αCD/5FU p.m. and βCD/5FU p.m.)

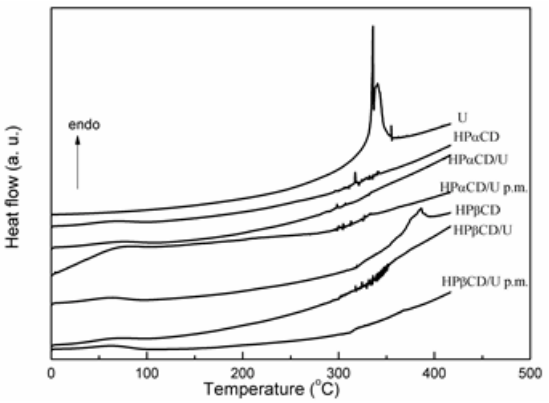

Fig. 3. DSC diagram for uracil (U), cyclodextrins (HPαCD and HPβCD), inclusion complex (HPαCD/U and HPβCD/U) and physical mixture (HPαCD/U p.m. and HPβCD/U p.m.)

For the αCD/5FU and βCD/5FU compounds, (fig. 2), HPαCD/U, HPβCD/U compounds (fig. 3), and HPαCD/5FU, HPβCD/5FU compounds respectively, (fig. 4), the thermal events of pure compounds was missing which indicating strong interactions of 5FU and host molecule. In the case of physical mixtures, (figs. 3, 4), we found that all thermal events of the pure compounds are still present for αCD/5FU p.m., βCD/5FU p.m., HPαCD/U p.m. HPβCD/U p.m., HPαCD/5FU p.m. and HPβCD/5FU p.m. respectively, which can suggest no presence of the 5FU molecule inside the cyclodextrin cavity.

The thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of the pure compounds and obtained complexes are shown in figure 5. In agreement with thermal profiles, all inclusion compounds show three main thermal events. Firstly, in temperature

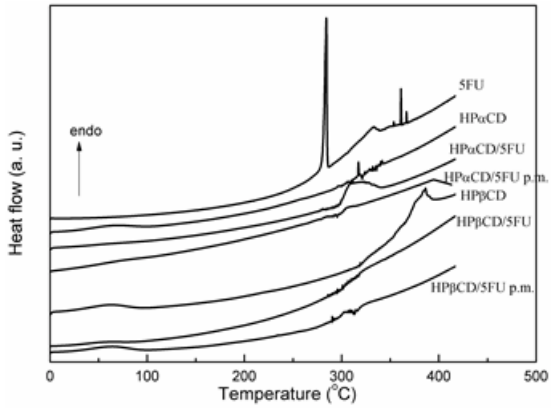

Fig. 4. DSC diagram for 5-fluorouracil (5FU), cyclodextrins (HPαCD and HPβCD), inclusion complex (HPαCD/5FU and HPβCD/5FU) and physical mixture (HPαCD/5FU p.m. and HPβCD/5FU p.m.)

Table 1
WEIGHT PERCENT (%) OF THE COMPLEXES RESULTED FROM DEHYDRATION AND GUEST LOSS PROCESSES

Inclusion complexes	Total mass loss by dehydration process (%)	Mass losses values of guest (%)
αCD/U	4.8	2.7
βCD/U	3.8	7.2
HPαCD/U	1.8	1.3
HPβCD/U	0.8	4.9
αCD/5FU	3.0	0.6
βCD/5FU	3.2	4.1
HPαCD/5FU	3.1	0.9
HPβCD/5FU	2.2	4.6

domain 30 – 130 C the thermogravimetric curves of CDs/U and CDs/5FU complexes show that the release of bound water at various energies took place in 2–3 endothermic stages, [8, 12-14]. Water-loss took place in two stages for αCD/U, αCD/5FU, βCD/U, βCD/5FU complexes and in three stages for HPαCD/U, HPαCD/5FU, HPβCD/U, HPβCD/5FU complexes. Secondly, very small amounts of guest molecule were released (this step occurred endothermic too) from inclusion compounds before the starting of the main thermal event [13,15]. Finally, for all analyzed complexes the main decomposition was occurred above 250°C and for all analyzed samples the decomposition step was followed in a large temperature domain by a running carbonization stage [16]. The percentage values of mass losses of guest and of total mass loss by dehydration process were presented in table 1.

Generally, the water molecules inside the CD's cavity are replaced by guest molecules when the complex was formed, thus the multi-step dehydration are induced by the guest content found in CD's cavity. We can conclude that the dehydration peaks located up to 100 °C are assigned to complexes with smaller U or 5FU content [15]. In figure 5b, the DTG curves of HPβCD/5FU, HPβCD/U, HPαCD/5FU and HPαCD/U show for the main thermal event a large and asymmetric curve which starts at a lower temperature than for complexes βCD/5FU, βCD/U, αCD/5FU and αCD/U, (fig. 5a and 5b). Further, the minimum temperature of the main thermal event was shifted toward a higher temperature for complexes between U or 5FU with 2-hydroxypropyl derivatives of α- and β- CD than for the complexes between U or 5FU with native CDs (fig. 5a and 5b). These facts proved there was a multi-step decomposition for HPβCD/5FU, HPβCD/U, HPαCD/5FU and HPαCD/U compounds, which means they have the greatest amount of guest inside the CD cavity and they are the most thermally stable.

The FTIR spectroscopy technique has been used for qualitative study to investigate the interaction of guests and CDs. Figure 6 shows the FTIR-ATR spectra of U/5FU, CDs and their 1:1 inclusion complexes, in the solid state. As one can see in figure 6, the spectra of the cyclodextrins inclusion complexes show variations compared with those of the corresponding pure U and 5FU [17]. In 5FU spectrum, the bands at 3120 cm^{-1}, 3064 cm^{-1}, 2929 cm^{-1} and 2826

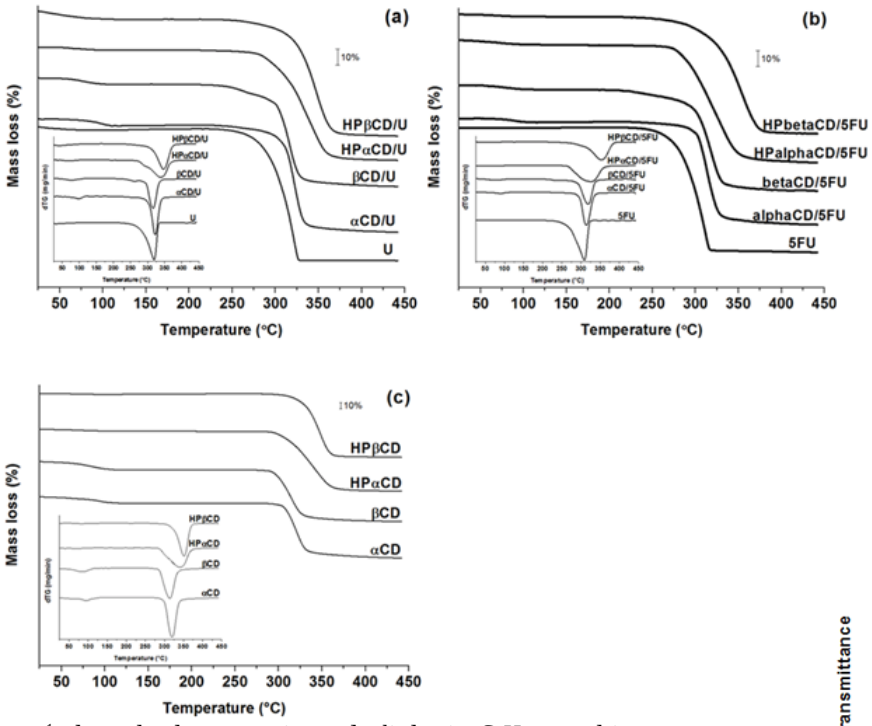

Fig. 5. TG and DTG (inset) curves of pure U and αCD/U, βCD/U, HPαCD/U, HPβCD/U inclusion complexes (a), pure 5FU, αCD/5FU, βCD/5FU, HPαCD/5FU, HPβD/5FU inclusion complexes (b), pure cyclodextrins (c)

cm^{-1} show both aromatic and aliphatic C-H stretching vibrations, a band at 1719 cm^{-1} is attributed to the imide group stretching of heterocyclic ring, the band at 1644 cm^{-1} is due to the -C=C- group stretching vibration, at 1428 cm^{-1} the band is due to N-H bending vibration, a band at 1243 cm^{-1} shows ring stretching vibrations and C-F stretching vibration was observed at 1222 cm^{-1} and 803 cm^{-1} respectively [18-20]. In the FTIR spectrum of U the principal absorption peaks are at 3088 cm^{-1} (N-H stretching band), 2985 cm^{-1}, 2933 cm^{-1} and 2821 cm^{-1} respectively, which could be due to both aromatic and aliphatic C-H stretching vibrations, 1712 cm^{-1} (C-N stretch vibrations), 1642 cm^{-1} (carbonyl stretching vibration), 1417 cm^{-1} (N-H in plane bending vibration), 1233 cm^{-1} and 821 cm^{-1} due to C-H stretching vibrations [21, 22].

The pure CDs spectra show a characteristic large band with the absorption maximum at 3288 cm^{-1} for αCD, at 3314 cm^{-1} for βCD, 3302 cm^{-1} for HPαCD and 3330 cm^{-1} for HPβCD. These bands were assigned to symmetric and anti-symmetric O-H stretching modes and are affected when complexation is done [14]. As can be seen in figure 6, the bands of pure guests in the region between 2800 cm^{-1} and 3200 cm^{-1} assigned to the aromatic ring were strongly affected upon complexation. In complexes spectra, some of the absorption characteristic peaks look depressed, broadened or displaying frequency shifts to higher wavenumbers [17, 23]. There are slight modifications detected in region of the -OH group stretching for the αCD/5FU, αCD/U, HPαCD/5FU and HP αCD/U systems. However, the band around 3300 cm^{-1} is shifted and broadened much more in case of HPβCD/5FU, HPβCD/U, βCD/U and βCD/5FU systems which for the encapsulation of the ligand is accomplished by involving a large number of hydrogen bonds [21].

The observed changes in the complexes spectra can be attributed to the interaction of the pyrimidine nucleus of U or 5FU (region at 1243–1428 cm^{-1}) with hydrophobic cavity of CD which indicates the inclusion complex formation for each considered system.

As one can see, the spectra of all inclusion complexes resemble that of pure CDs, another indication for encapsulation of guest molecule into the CD cavity. Therefore, in agreement with the proposed molar ratio of host:guest systems, it is expected that the FTIR

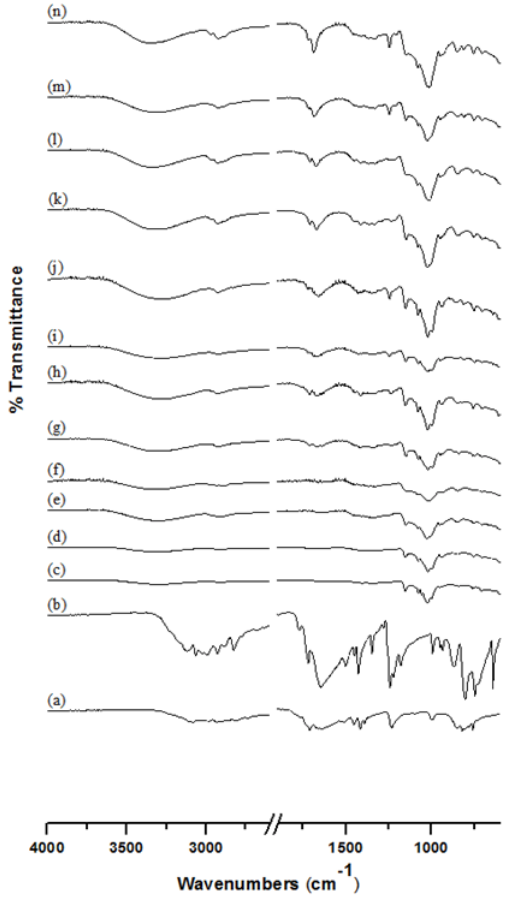

Fig. 6. FT-IR spectra of (a) - Uracil (U), (b) - 5-fluoro-uracil (5FU), (c) - αCD, (d) - βCD, (e) - HPαCD, (f) - HPβCD (pure compounds) and (g) - αCD/U, (h) - βCD/U, (i) - αCD/5FU, (j) – βCD/5FU, (k) - HPαCD/U, (l) - HPβCD/U, (m) - HPαCD/5FU, (n) - HPβCD/5FU (inclusion complexes)

spectroscopic data to be in concordance with the results found on the TG/DTG and DSC analyses.

The shape and surface morphologies of the pure compounds and its inclusion complexes were further evaluated by SEM method. For native compounds the morphology resulted from SEM images was already presented in literature [24, 25]. In figure 7 were shown the SEM data of the prepared complexes in 1:1 molar ratio of the host and guest. As can be seen, for αCD/U, HPαCD/U systems the small U crystals raised from the CD texture, while for the αCD/5FU, βCD/5FU and βCD/U complexes one can see particles of αCD or βCD embedded with ligand particles. The morphology observed for the αCD/U, HPαCD/U, βCD/U, αCD/5FU and βCD/5FU systems indicates an incomplete inclusion complex formation [26], or an excess mass of pure component. In contrast, a clear amorphous structure was observed for inclusion compounds of HPβCD with U and 5FU and for HPαCD with 5FU, (fig. 7 g), h), f)), which for the two parent components were indistinguishable, thus suggesting a

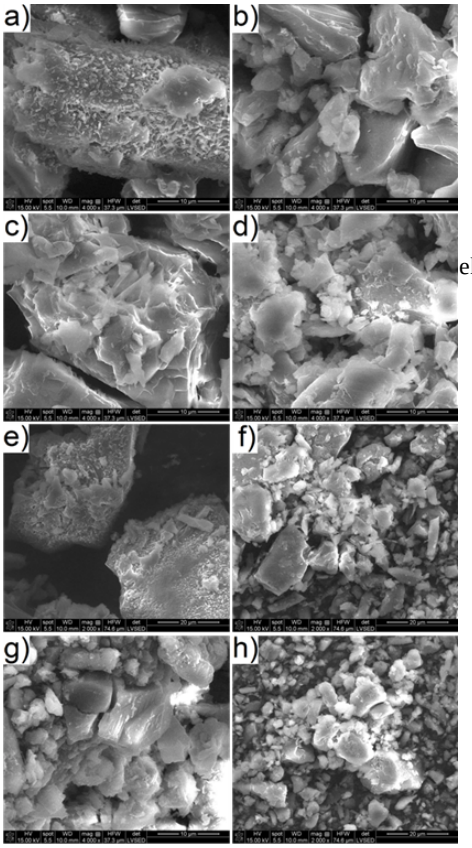

Fig. 7. Scanning electron micrographs of inclusion complexes (a) - αCD/U, (b) - αCD/5FU, (c) - βCD/U, (d) - βCD/5FU, (e) - HPαCD/U, (f) - HPαCD/5FU, (g) - HPβCD/U, (h) - HPβCD/5FU (inclusion complexes) at 25 ± 1 °C

complete encapsulation of the ligand into the CD cavity [27]. For the analyzed samples, there is a clear change in crystallization state of the native materials and the resulted complexes. In this study, SEM data support the presence of a single component in the obtained products and provide available information about the efficiency of the complexation process [25].

Conclusions

The formation of the inclusion complexes of guest U or 5FU with α- , β- cyclodextrins and its 2-hydroxypropyl derivatives have been analyzed by using DSC, TG, FTIR and SEM. The obtained results suggest that all the cyclodextrin based inclusion compounds synthesized by melting in solution method in a molar ratio of 1:1 were successfully prepared. Considering the obtained data, it was clearly demonstrated that the guest (U or 5FU) is encapsulated inside the macrocyclic cavity of CD. Thus, we can conclude that the size of the truncated cone of the CD has a significant influence on the complexation interaction because the diameter of each used CD is large enough to embed the planar molecule of the U or 5FU. However, the thermal stability of the complexes is also influenced by the flexibility of the CD ring which reducing the rejection of the guest and favor the hydrogen bonds formation. Comparing the results, we can say that the inclusion complexes formed between U or 5FU with βCD and HPβCD were found to be the more stable than the complexes formed with α- and HPα- CD. Moreover, the 5FU molecule interacts weaker with α- and HPα- CD and stronger with β- and HPβ- CD than the U molecule. Correlating the experimental data it can be established that the size of the CD cavity more than the presence of hydroxypropyl groups in CD molecule has a considerable influence on the complexation process.

Acknowledgements: The Romanian Academy as well as E.U. (ERDF) and Romanian Government support for acquisition of the research infrastructure under POS – CEE 2.2.1 Project INFRANANOCHEM/2007 - 2010 is acknowledged.

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Manuscript received: 22.03.2017