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COMPARATIVE PHYSICOCHEMICAL INVESTIGATION OF THE INCLUSION COMPOUNDS OF CYCLODEXTRINS WITH ARGININE AND HISTIDINE STEREOISOMERS

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The inclusion complexes of *α*-, *β*-cyclodextrin and 2-hydroxypropyl-*α*-cyclodextrin with two amino acid stereoisomers (L-, D-arginine and L-, D-histidine) were studied by using differential scanning calorimetry, thermogravimetry, Fourier transform infrared spectroscopy, and scanning electron microscopy methods. The solid inclusion compounds were prepared in a 1:1 molar ratio of the host and guest using the co-precipitation method. The pH measurements and structural visualization of complexes were carried out. The obtained results proved the formation of the complexes and revealed that the size and the symmetry of the cyclodextrin (CD) and also the structure and flexibility of the amino acid molecule had a significant influence on the complexation interaction. The correlation of the experimental data shows that *β*CD has a preference to form more stable complexes with levogir isomers of amino acid than *α*CD and 2-hydroxypropyl-*α*-cyclodextrin. The complexation of the amino acids isomers was accomplished by partial inclusion of the guest molecule in the CD cavity, and it was observed that CDs could better discriminate between histidine isomers than between arginine isomers.

Keywords: cyclodextrin; histidine; arginine; inclusion complex; thermal analysis

КОМПАРАТИВНО ФИЗИЧКО-ХЕМИСКО ИСТРАЖУВАЊЕ НА ИНКЛУЗИВНИ СОЕДИНЕНИЈА НА ЦИКЛОДЕКСТРИНИ СО СТЕРЕОИЗОМЕРИ НА АРГИНИН И ХИСТИДИН

Со примена на диференцијалната калориметрија за скенирање, термогравиметријата, Фуриеовата трансформна инфрацрвена спектроскопија и методи на електронска микроскопија за скенирање беа проучувани инклузивните комплекси на α-циклодекстрин, β-циклодекстрин и 2-хидроксипропил-α-циклодекстрин со два стереоизомера на аминокиселини (L-, D-аргинин и L-, D-хистидин). Цврстите инклузивни соединенија беа подготвени во моларен сооднос 1:1 на домаќинот и гостинот користејќи го методот на коталожење. Беа извршени мерења на pH и структурна визуализација на комплексите. Добиените резултати докажаа формирање на комплексите и покажаа дека големината и симетријата на циклодекстринот (CD), како и структурата и флексибилноста на молекулата на аминокиселината имаат значително влијание врз интеракцијата на комплексирањето. Корелацијата на експерименталните податоци покажува дека βCD преферира да формира постабилни комплекси со левогирски изомери на аминокиселините отколку αCD и 2-хидроксипропил-α-циклодекстрин. Комплексирањето на изомерите на аминокиселините беше постигнато со делумно навлегување на гостинската молекула во празнината на CD. Исто така беше забележано дека CD подобро разликуваат помеѓу изомерите на хистидин, отколку помеѓу изомерите на аргинин.

Клучни зборови: циклодекстрин; хистидин; аргинин; инклузивни комплекси; термичка анализа

1. INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides containing six or more D-(+)-glucopyranose units.[1] The interior of the CD torus is relatively hydrophobic due to the high electron density of the glycosidic oxygens present in the cavity, while on the outside, the CD torus is relatively hydrophilic because of hydroxyl groups.[2,3] It is well known that their unique structure allows them to form host-guest complexes with various substances through weak interactions like hydrophobic interactions, Van der Waals forces, and hydrogen bonding.[1,2,4] Besides the improvement of the stability and solubility of the substances, understanding and controlling host-guest interactions is a fundamental aspect of designing new materials and developing innovative technologies in many scientific disciplines.

Nowadays, CDs continue to be the subject of numerous research studies aimed at exploring their potential applications in more specific areas. The interaction of CDs with specific amino acid residues contained in proteins or polypeptides is of significant importance due to its relevance in various fields, including pharmaceuticals, food science, and biochemistry. Some key aspects of this interaction are related to protein's properties: this interaction results in several benefits such as decreased protein aggregation and enhanced shelf-life of therapeutic proteins, improved thermal and proteolytic stability, enhanced refolding yields, and increased bioavailability.[5,6] For instance CDs can act as artificial chaperones and some CDs can function as chaotropic agents that delay protein-protein interactions, resulting in delayed folding in solution. Also, these properties could have implications in enzyme modulation, protein folding mechanisms associated with misfolding diseases, therapeutic peptides containing amino acids, and taste masking in pharmaceuticals.[5,7,8]

Further, CDs are the most frequently used chiral selectors because they have many of the desirable features to accomplish proper enantioselectivity for a wide range of substances, including amino acids.[9,10] Features, such as amino acid fit to the cavity and its hydrogen bonding potential, CD crystal packing arrangement, and the interaction with the solvent used in preparation combine to play a significant role in chiral and molecular recognition. There are some reference studies in which structures and properties are determined for CD complexes formed with both amino acid enantiomers separately and for the racemate.[11–13] Furthermore, the formation of cyclodextrin-amino acid complexes has been utilized for enantioseparations and chiral separations in analytical techniques, providing a means to distinguish between different forms of amino acids.[14,15] The smaller cavity size of α-cyclodextrin (αCD) is crucial for their selectivity and efficiency in incorporating amino acids. The size of the cavity determines the type and number of amino acids that can be included. Smaller cavity sizes ensure a more snug fit and better accommodation of smaller amino acid residues.[16] Larger amino acids with more hydrophobic side chains (also, aromatic amino acids like phenylalanine, tyrosine, and tryptophan) tend to interact more strongly with β-cyclodextrins (βCDs).[17–19] For example, relevant discrimination was observed for the αCD inclusion complex with D-/L- tryptophan in the solid state, whereas there was no apparent discrimination for the βCD inclusion complex with D-/L-tryptophan.[20] It was found that both the α- and β-CDs form 1:1 stoichiometry of host-guest inclusion complexes with L-, D-amino acids at both low and high pH.[21–23] The essential amino acids arginine (Arg) and histidine (Hist) play various roles in the human body. Arg acts as a precursor for the production of nitric oxide and is an important substrate in the synthesis of many substances in the human body. It was demonstrated that in proteins there are arginine-rich domains that often play important roles in various cellular processes, such as protein-protein interactions, nucleic acid binding, and more.[24–26] Hist is often involved in the catalytic mechanism of serine proteases. It acts as a general base, abstracting a proton from the serine hydroxyl group, which enhances the nucleophilicity of the serine and allows it to attack the peptide bond in the substrate.[27,28] Besides their biological importance, some papers show special features which extend the use of Arg and Hist by complexation with cyclodextrins.[29] Although, the ^{1}H NMR (Proton Nuclear Magnetic Resonance) spectroscopy study confirms the formation of the inclusion complexes for the levo-form or Arg and Hist with natural CDs by observing the chemical shift of the protons of the CD molecules. The surface tension and conductivity studies provide further evidence for the formation of these complexes, with a stoichiometry of 1:1.[29] Arg complexes with CDs were characterized using various techniques, including molecular modeling analysis and isothermal titration calorimetry measurements, which demonstrated that Arg has a higher affinity for 2-hydroxypropyl-β-cyclodextrin (HPβCD) than for βCD.[30] This work comes to complete the already existing information. Herein, the host-guest inclusion complexes of natural amino acids, namely, L-, D-arginine (*L*Arg, *D*Arg) and L-, D-histidine (*L*Hist,

*D*Hist) as guests with *α*CD, 2-hydroxypropyl-*α*-cyclodextrin (HP*α*CD), and *β*CD was accomplished as a qualitative study for the solid powders of the synthesized complexes by using differential scanning calorimetry (DSC), thermogravimetry (TG), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) methods.

2. EXPERIMENTAL

2.1. *Preparation of the inclusion complexes*

*D*Arg (purity 99 %), *L*Arg (purity 98 %), *D*Hist (purity 99 %), *L*Hist (purity 99 %), *α*CD (purity 98 %), HP*α*CD (purity 99 %), and *β*CD (purity 97 %) were purchased from Sigma Aldrich Chemical Company and used in preparations without further purification. The preparation of solid-state inclusion compounds in a 1:1 molar ratio of host and guest was completed using a co-precipitation method. The host molecules – *α*CD, *β*CD, and HP*α*CD – and the amino acid stereoisomers of Arg and Hist were dissolved in double distilled water. The guest solution was then added dropwise to the CD solution. The resulting amino acid/CD mixtures were stirred (600 rpm) for 6 h at room temperature, then dried under vacuum at 40 °C. The resulting solid white powders of *L*Arg/*α*CD, *D*Arg/*α*CD, *L*Hist/*α*CD, *D*Hist/*α*CD, *L*Arg/HP*α*CD, *D*Arg/HP*α*CD, *L*Hist/HP*α*CD, *D*Hist/HP*α*CD, *L*Arg/*β*CD, *D*Arg/*β*CD, *L*Hist/*β*CD, and *D*Hist/*β*CD complexes were used in the investigations.

2.2. *Methods*

The pH measurements were recorded with an accuracy of ±0.01 pH units using a Thermo Scientific Orion 5-Star Plus benchtop meter with model 9107BN Triode 3-in-1 pH/automatic temperature compensation probe. For the pH determination, stock solutions at constant concentrations (10^{-4} M) of amino acid isomers and CDs were prepared (using double distilled water as solvent) and mixed by varying the mole fraction of each component from 0 to 1. The pH measurements were done for the pure components and the mixture solutions by maintaining the temperature of the samples at 298.15 ± 0.5 K. The solid powders of the complexes formed between CDs with Arg and Hist amino acids were further qualitatively investigated by the different methods. The DSC and the TG data of the inclusion compounds and the pure substances were characterized using a TG analyzer coupled with DSC (Setaram Setsys Evolution 17) in open alumina crucibles of 100 μl volume. The calorimeter was calibrated using the recommended standards of indium (ΔH_{fus} = 28.46 J g^{-1}). The sample masses were between 1 mg and 2 mg. The measurements were performed at a heating rate of 10 °C min^{-1} in a flowing argon atmosphere (16 ml min^{-1}). FTIR spectral data of pure compounds and inclusion complexes were recorded at room temperature by Nicolet iS10 FTIR Spectrometer covering the range of 4000 to 600 cm^{-1}. The spectra were acquired with an average of 32 scans with a spectral resolution of 4 cm^{-1} in attenuated total reflectance (ATR) mode. The morphology of the samples was investigated by SEM using a high-resolution microscope, FEI Quanta 3D FEG model, operating at 15 kV, in low vacuum mode with a low vacuum secondary electron detector (LVSED). Sample preparation was minimal and consisted of immobilizing the material on a double-sided carbon tape, without coating.

3. RESULTS AND DISCUSSION

3.1. *pH measurements and structural visualization of complexes*

pH measurements were done to study the behavior of Arg and Hist isomers in the presence of different types of CDs and the obtained results are presented in Figure 1. For the temperature of 298.15 K, it was found that the pH of Arg and Hist in the presence of different CDs solutions was in the range of 6.4 to 8.6 and 6.3 to 8.0, respectively. The pH values of the mixture solutions decreased with the increase of CD concentration until the molar fraction of 0.5 was reached and did not change at high CD concentrations. Thus, it can be assumed that the evident change of the slopes (Fig. 1) at around a molar fraction of 0.5 may be due to a predominant 1:1 stoichiometric ratio of the inclusion complexes.[22,23,29]

The increase in CD concentration causes pH modification in the solution environment which occurs in the presence of many possible Arg or Hist species. In Figure 2, the percentage of microspecies distribution of Arg and Hist in aqueous solutions over the pH range between 6 and 9 and the corresponding structures are depicted. As can be seen, Arg(1), Hist(1), and Hist(3) are the main ionic forms that could interact with the CDs.

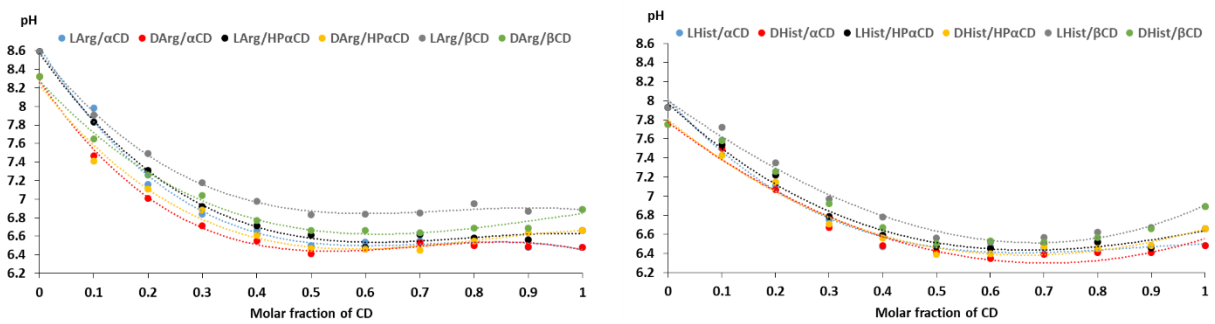

Fig. 1. Variations in the pH of amino acid/CD mixtures with molar fraction of the CD for Arg isomers with CDs (left panel) and Hist isomers with CDs (right panel)

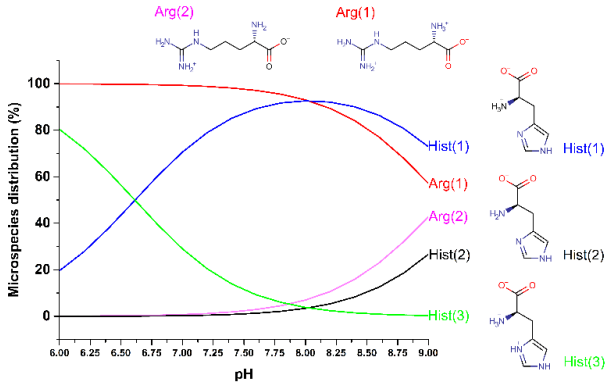

Fig. 2. Percent (%) microspecies distribution of Arg and Hist in aqueous solutions over the pH range between 6 and 9

The solid powders of CD complexes were obtained by the co-precipitation technique, which is generally used for hydrophobic substances.[31] Nevertheless, the binding interactions of CD, even with water-soluble molecules, are spontaneous and favored.[32,33] Arg and Hist are both hydrophilic amino acids containing hydrophobic parts. Hist is more hydrophilic than Arg, but Arg is more flexible than Hist.[34,35] Accordingly, the presence of the hydrophobic part of the amino acid promotes the interaction with the CD molecule. Furthermore, the flexibility of the amino acid enhances its ability to properly position its hydrophobic part into the CD cavity, thereby strengthening the hydrophobic interactions and adding some possible H-bonds by interacting with –OH groups on the CD's rims. So, no special preparation techniques are needed because the solubility of Arg and His does not affect the spontaneous complex formation with CD.[33] However, the encapsulation of the hydrophobic part of the amino acid is an entropy-driven process as it requires desolvation while releasing water molecules from the CD cavity.[36,37] The co-precipitation technique initially deals with the hydration of the amino acid and the CD molecules. Thus, the presence of water molecules prevents the association of two identical units and creates a barrier that hinders their complexation interaction. Further, by gently heating the mixture solution, in the stirring stage of the preparation, the molecules gradually lose their hydration shell allowing for a proper complexation interaction.[38,39] Nevertheless, the most hydrophobic part of the amino acid must be settled into the CD's cavity. According to literature data, *α*CD and *β*CD internal volume (cavity) of the geometric properties of the amino acids are suitable for the CD cavity. The small cavity of *α*CD has a good fit for amino acids, especially for the nonaromatic amino acids, while the *β*CD cavity could better accommodate any type of amino acid.[17–19,23]

In order to visualize the molecular structure of the inclusion compounds, the equilibrium geometries of Arg/*α*CD, Hist/*α*CD, Arg/*β*CD, and Hist/*β*CD are shown in Fig. 3. The presented assemblies do not consider water presence and the ionization states, as well as the tautomers of imidazole in Hist which were not considered in this approximation. The optimization geometries of the pure molecules and the amino acid/CD structures were achieved by generalized gradient approximation (GGA) with exchange-correlation functional PBE (Perdew-Burke-Ernzerhof). The starting molecular assembly was created assuming a 1:1 stoichiometry model by positioning the amino acid-optimized molecule in two ways into the *α*CD cavity (also with geometry optimized) and considering the condition of a superposing of the center of mass of the two molecules. The first way (in Fig. 3, left side of each panel top view and side view) occurs when the guest molecule is oriented along the *α*CD cavity with the carboxylic group of the guests pointing toward the narrow rim of the *α*CD, and the second way (in Fig. 3, right side of each panel top view and side view) is obtained through 180° rotation of the molecule by positioning the carboxylic group of the guest toward the wider rim of the *α*CD molecule.

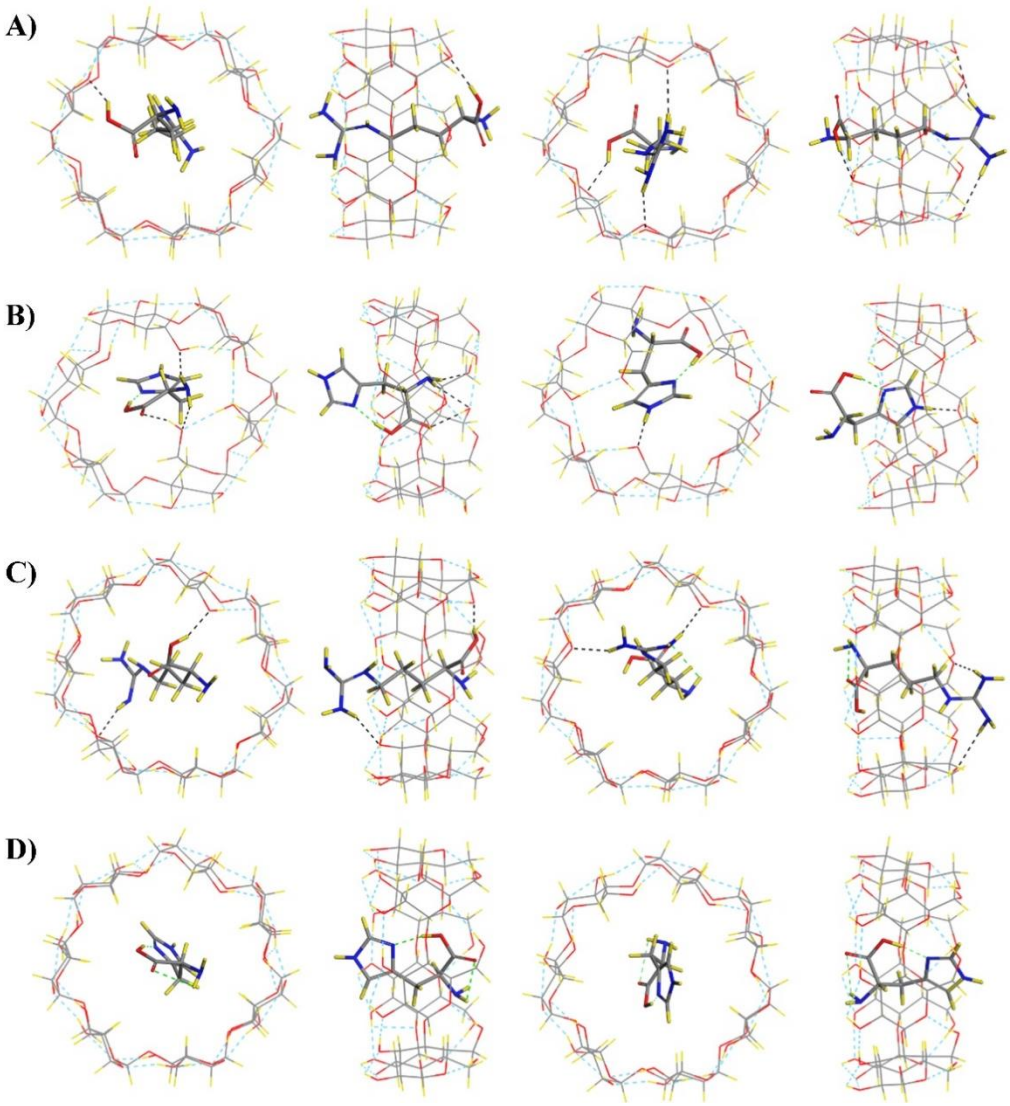

Fig. 3. Spatial representation of the equilibrium geometries of two types of 1:1 host-guest assemblies – top view (through the primary hydroxyl rim of CD) and - side view (primary hydroxyl rim of CD positioned to right side) of Arg/*α*CD (Panel A), Hist/*α*CD (Panel B), Arg/*β*CD (Panel C), Hist/*β*CD (Panel D), browsing horizontally. Type one of the 1:1 complex occurs by pointing the carboxylic group of the amino acid toward the narrow rim of the CD (left side of each panel top view, and side view) and type two shows the carboxylic group of the amino acid pointed toward the wider rim of the CD molecule (right side of each panel, top view and side view). The color correspondence is: blue for N atoms, grey for C atoms, yellow for H atoms, and red for O atoms. Hydrogen bonds are indicated by dotted lines: black for H bonds between amino acid and CD, light green for intermolecular H bonds in amino acid molecule, and cyan for CD's H bonds.

As can be seen in Figure 3, the amino acids were not expelled from the CD cavity. A high displacement of the mass center of amino acids happened for *α*CD because of the cavity size related to amino acid molecular dimensions, so Hist and Arg had a better fit in the *β*CD cavity than in *α*CD cavity. Accordingly, to *α*CD physical dimensions, the amino acids form more H bonds with *α*CD than with *β*CD because the amino acid functional groups suitable for H bond formation are closer to the hydroxyl groups on the *α*CD's rims. Hist fits better in the *β*CD cavity than Arg, but it forms fewer H bonds with *β*CD than Arg. This can be due to the selected conformer structure which tends to form more intramolecular bonds than Arg (the green dotted line in Fig. 3). Due to its hydrocarbon chain, Arg molecules remain flexible and this can impact its ability to participate in hydrogen bonding with CD. For the complexes formed between Arg and Hist with cyclodextrins, similar spatial representations were confirmed based on ^{1}H NMR studies in aqueous solution.[29]

Further, the synthesized powders of solid inclusion complexes of L-,D-Arg/Hist with CDs were investigated by different techniques to establish the efficiency of the complexation and to highlight some particularities of the obtained complexes.

3.2. *Thermal analysis*

TG/DSC analyses are important thermal analysis methods usually used to investigate the interactions that can occur in a host–guest complex. A comparative thermal analysis of pure CDs (Fig. 4), free L-, D-Arg/Hist amino acids and complexes (Fig. 5) was performed.

The thermal behavior of the amino acids and CDs varies according to the experimental conditions used in investigations, therefore the thermal parameters of the main thermal processes of the pure substances used in this study are shown in Table 1.

The resulting data (Table 1 and Fig. 5) show that the enantiomers pairs (*L*Arg, *D*Arg and *L*Hist, *D*Hist) are similar. The difference of 1 ºC between the T_m temperatures is caused by the different morphologies of the samples due to the preparation method of the enantiomer. In the case of the amino acid isomers, such thermal differences were already reported in the literature. The thermal behavior of complexes (Fig. 5) is described by several endothermic effects observed in the temperature range between 25 °C and 400 °C, typically attributed to the dehydration process, followed by a thermal effect due to the rearrangement of chemical bonds and melting or a melting-decomposition event.[20]

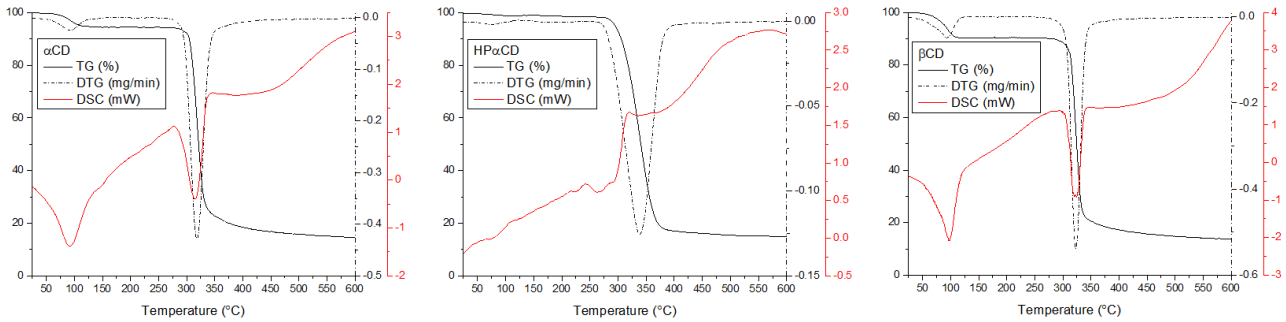

Fig. 4. DSC (solid red line), TG (solid black line), DTG (dash-dot line) curves of the pure CDs

T a b l e 1

Thermal parameters (the onset-, maximum-, offset-temperatures and corresponding enthalpy) of degradation processes of pure compounds from DSC data at 10 K min^{-1}

Sample	T_{on} [°C]	T_m [°C]	T_{off} [°C]	ΔH [J/g]	Physical phenomenon
	69.5	100.1	129.5	9.9	Dehydration
***L*Arg**	208.9	216.6	222.6	45.2	Melting
	241.9	245.6	254.1	371.9	Decomposition
	22.6	83.3	123.2	131.5	Dehydration
***D*Arg**	211.6	215.3	221.1	23.5	Melting
	239.7	243.2	251.8	333.6	Decomposition
***L*Hist**	268.2	282.9	287.4	570.1	Melting
***D*Hist**	277.1	281.9	286.5	1110.3	Melting
	54.5	92.1	126.4	168.5	Dehydration
***α*CD**	137.1	148.7	157.8	2.18	Solid-solid transition
	280.4	315.7	336.7	211.9	Melting-decomposition
	41.6	82.6	104.9	28.2	Dehydration
	118.7	126.3	135.8	0.5	Dehydration
HP*α*CD	217.3	227.9	234.3	3.2	Premelting
	247.7	266.8	281.1	21.8	Melting-decomposition
	284.5	298.4	309.1	36.9	Decomposition
	58.7	96.7	118.6	248.2	Dehydration
***β*CD**	274.3	282.3	290.1	1.6	Melting
	305.3	322.7	338.6	176.8	Decomposition

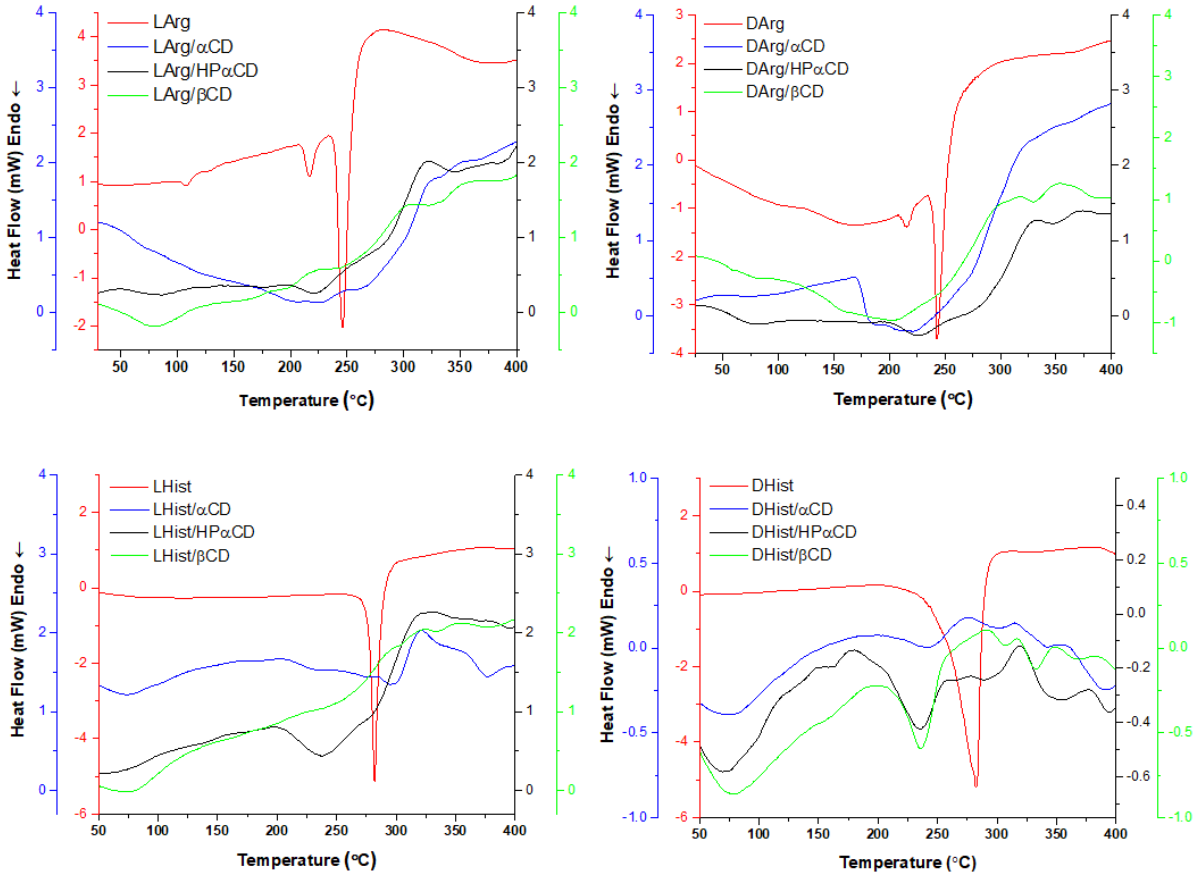

Fig. 5. DSC curves of pure L-, D-amino acids and of the inclusion complexes

In the case of the complexes containing HP*α*CD and for complexes *D*Hist/*α*CD and *D*Hist/*β*CD, the characteristic melting curves of the amino acids are strongly reduced, broadened, and shifted to a lower temperature. These shifts revealed that the complex formation involved the molecular arrangement of the amino acid in the solid caused by the reduction of drug crystallinity. This may be attributed to the presence of less binding between the pure components in the complex and/or partial inclusion of the amino acid molecule.[40] Also, the reduction in the fusion enthalpy of amino acid after inclusion in the CD cavity can be seen. However, the parameters of the melting event of the complexes containing HP*α*CD and for complexes *D*Hist/*α*CD and *D*Hist/*β*CD could be easily visualized in Figure 6.

For *D*Arg/*α*CD, L-,D-Arg/*β*CD, and *L*Hist/*β*CD complexes there are no endothermic events corresponding to the melting process of the amino acid. Disappearance of the endothermic effect characteristic of pure amino acid suggests that the complex has been formed by deep inclusion of the guest in the CD cavity, this phenomenon being considered as indicative of a stronger interaction between CD and stereoisomer in the solid state.[41]

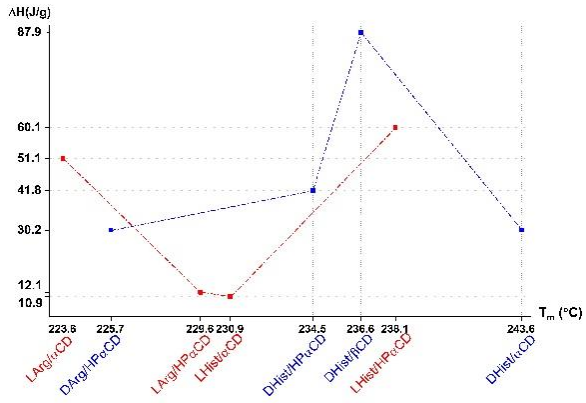

Fig. 6. Transition temperature and corresponding enthalpy of the melting endotherm observed from DSC data of the inclusion complexes of CDs with levogyr isomers of Arg and Hist (red) and with dextrogyr isomers of Arg and Hist (blue).

In Figure 7, the TG/DTG curves for pure amino acids and for the inclusion complexes are shown. In the temperature domain 30 – 130 ºC, the thermogravimetric curves show that L-,D-Arg/CDs and L-,D-Hist/CDs complexes eliminate small quantities of their water content in 2 or 3 endothermic stages, including CD-bound water at various energies.[42–45]

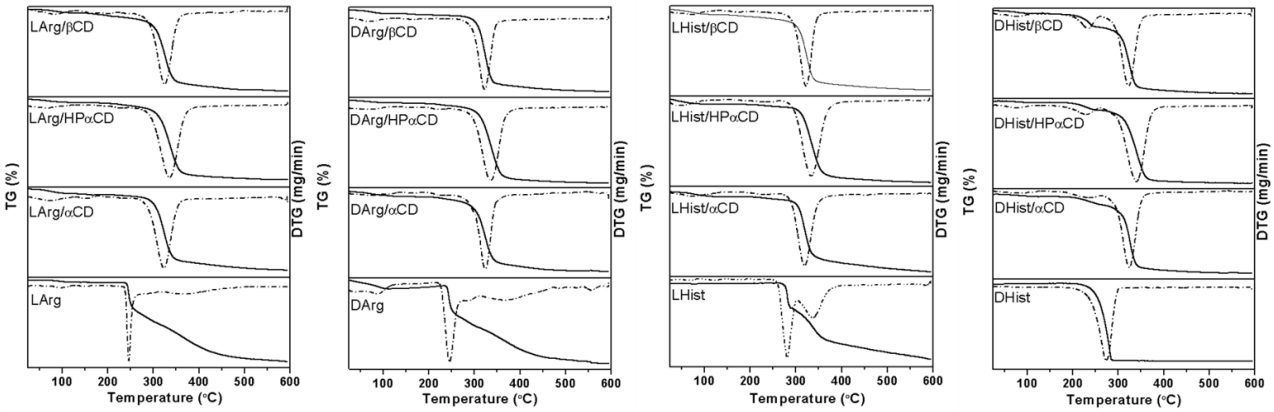

Fig. 7. TG and DTG (dash dot line) curves of pure amino acids Arg and Hist and of the *L*Arg/*α*CD, *L*Arg/HP*α*CD, *L*Arg/*β*CD, *D*Arg/*α*CD, *D*Arg/HP*α*CD, *D*Arg/*β*CD, *L*Hist/*α*CD, *L*Hist/HP*α*CD, *L*Hist/*β*CD, *D*Hist/*α*CD, *D*Hist/HP*α*CD, and *D*Hist/*β*CD inclusion complexes

The water molecules inside CD's cavity were replaced by guest molecules when the complex was formed. Thus, the multi-step dehydration induced by the guest content found in CD's cavity, and the dehydration peaks located up to 100 °C, are assigned to complexes with smaller amino acid content.[46] In agreement with thermal profiles (Fig. 7) it can be observed that very small amounts of the guest molecule were released (this step occurred endothermically too) from their inclusion compounds before the decomposition process took place.[44,46] For the analyzed complexes, the main decomposition step occurred above 250 ºC and was followed by a running carbonization stage in a wide temperature domain.[41]

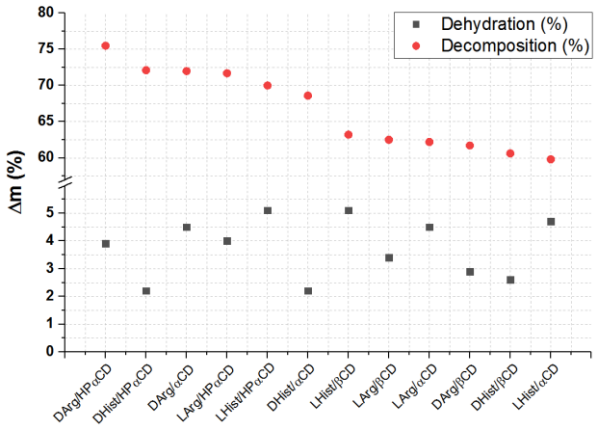

Fig. 8. Mass loss of the decomposition process (red points) and mass loss of the dehydration process (black square) of the inclusion complexes of CDs with L- / D-Arg and L- / D-Hist amino acids

The DTG curve of the main thermal event shows a sharp and symmetric peak for the complexes which points to a lower temperature for the complexes formed with *α*CD than for complexes formed with HP*β*CD and *β*CD. For all of the inclusion compounds the percentage values of total mass loss by the dehydration process and for the decomposition process are depicted in Figure 8.

3.3. *ATR FTIR analysis*

Figure 9 and Figure 10 show the FTIR-ATR spectra of L-, D-Arg, L-, D-Hist, and CD along with their 1:1 inclusion complexes, in the solid state. As one can see, the pure CD spectra show a characteristic large band with an absorption maximum at 3288 cm^{-1} for *α*CD, at 3314 cm^{-1} for *β*CD, and at 3302 cm^{-1} for HP*α*CD. These bands were assigned to symmetric and anti-symmetric O–H stretching modes and are affected when complexation is done through hydrogen bonding interactions.[45,47] In the FTIR spectra of inclusion complexes, the band at around 3300 cm^{-1} is strongly affected due to the involvement of the N–H group of the amino acid and O–H of the CD. As can be seen in Figures 9 and 10, the spectra of the complexes *L*Arg/*α*CD, *L*Arg/*β*CD, *L*Hist/*α*CD, *L*Hist/HP*α*CD, *L*Hist/*β*CD, *D*Arg/*α*CD, *D*Arg/*β*CD, *D*Hist/*α*CD, *D*Hist/HP*α*CD, and *D*Hist/*β*CD are shifted in the range of valence *v* (O–H) vibrations by about 15 cm^{-1} to 20 cm^{-1} towards lower frequencies, which means that the hydrogen bonding provides an important contribution to the formation of the inclusion complexes.[48,49] For *L*Arg/HP*α*CD and *D*Arg/HP*α*CD complexes the frequency position of valence *v* (O–H) vibrations are shifted by about 30 cm^{-1} towards higher frequencies compared to the position of the similar band in the spectrum of CD. The shift of the O–H stretching peak to a higher wavenumber is often attributed to more intense associations by hydrogen bonding upon the formation of complexes with HP*α*CD.[50] The signal in the CD spectra at around 2920 cm^{-1} (C–

H stretch) is affected by shifting towards higher frequencies for complexes *L*Arg/*α*CD, *D*Arg/*α*CD, *L*Hist/*α*CD, *D*Hist/*α*CD, *L*Arg/*β*CD, *D*Arg*β*, *L*Hist/*β*CD, and *D*Hist/*β*CD, and are affected by shifting to lower frequencies for complexes *L*Hist/HP*α*CD, *D*Hist/HP*α*CD, *L*Arg/HP*α*CD, and *D*Arg/HP*α*CD.

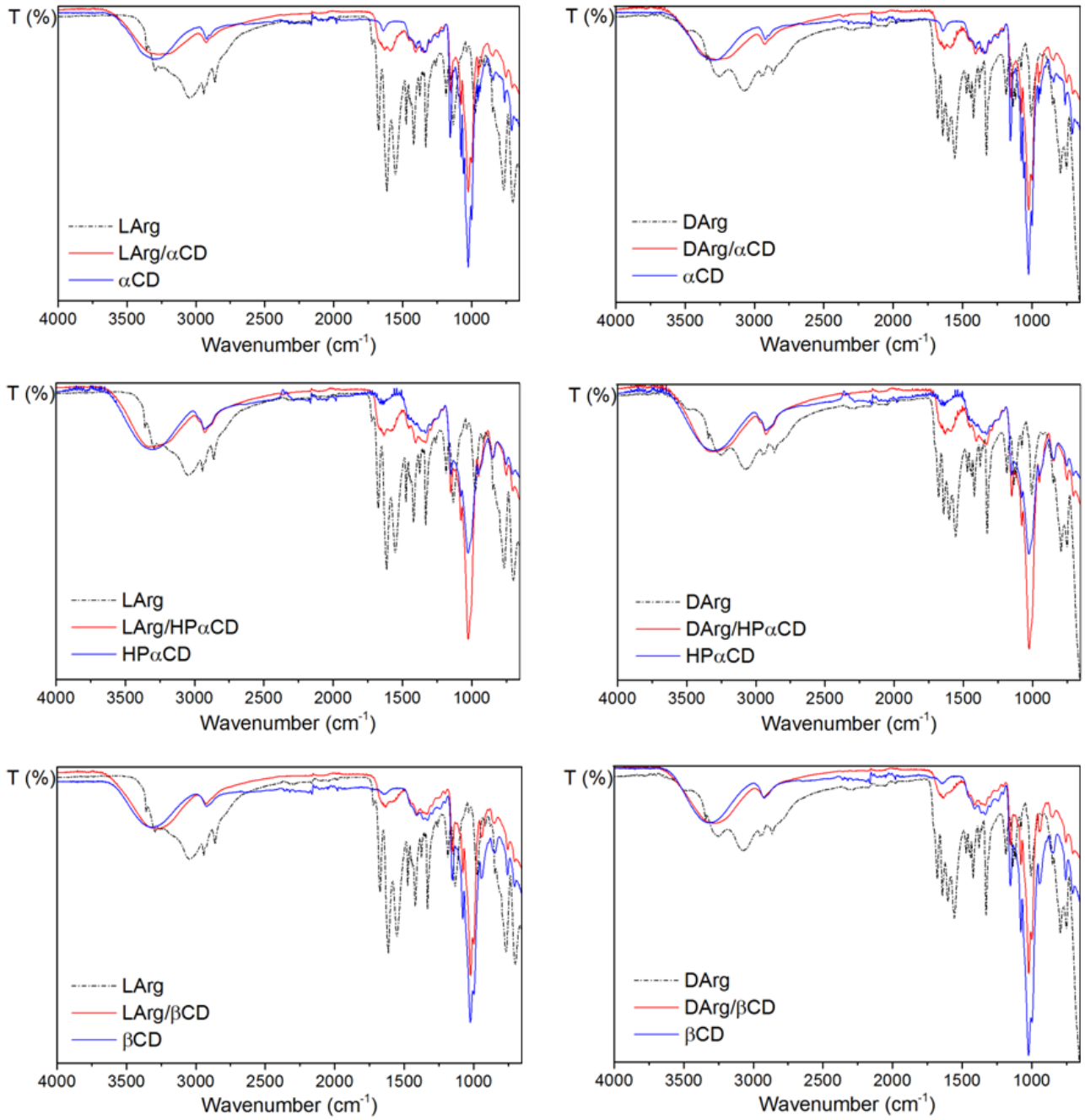

Fig. 9. FTIR spectra of solid powders of pure *L*Arg, *D*Arg, CDs and *L*Arg/*α*CD, *L*Arg/HP*α*CD, *L*Arg/*β*CD, *D*Arg/*α*CD, *D*Arg/HP*α*CD, and *D*Arg/*β*CD inclusion complexes

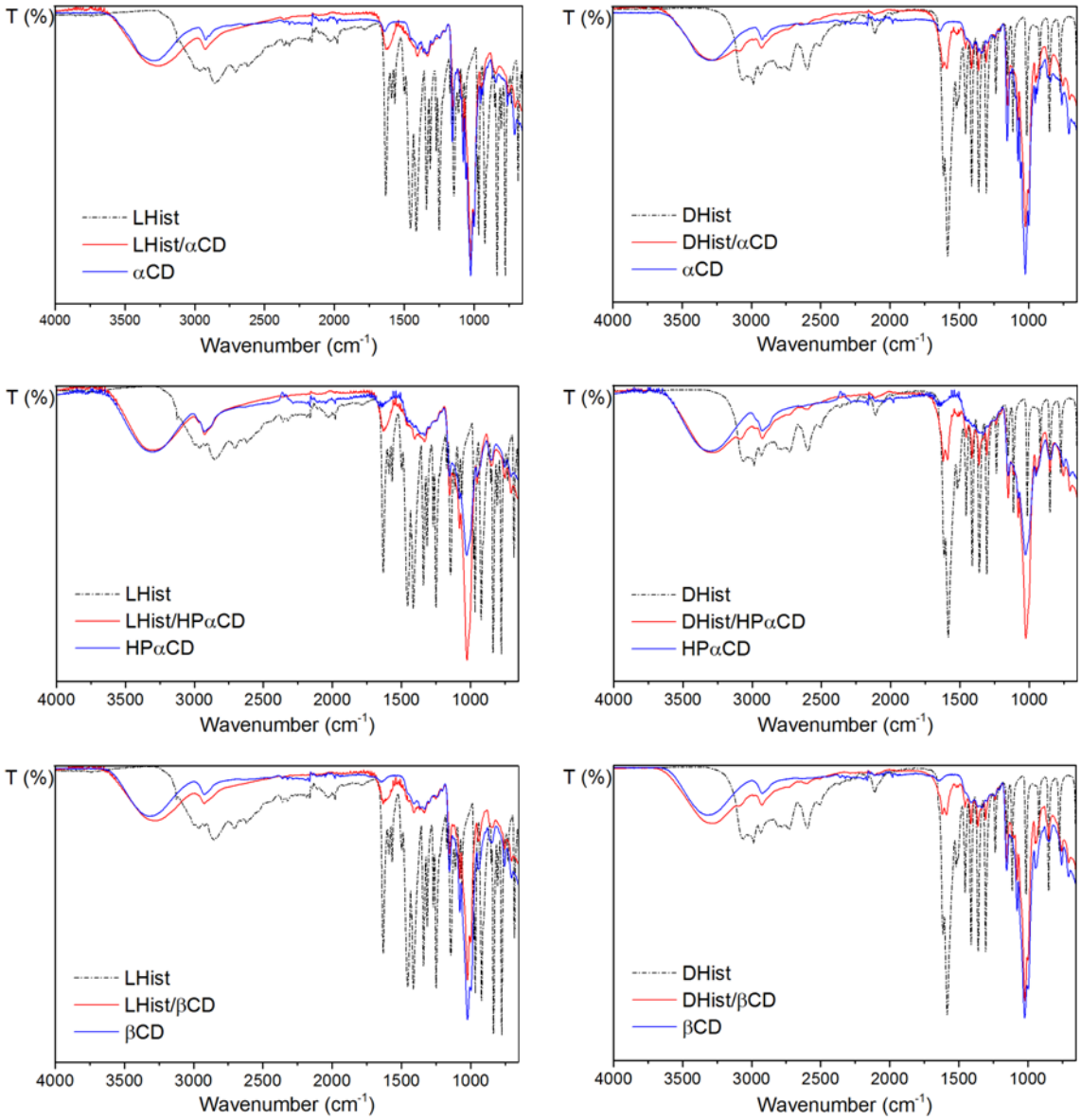

Fig. 10. FTIR spectra of solid powders of pure *L*Hist, *D*Hist, CDs and *L*Hist/*α*CD, *L*Hist/HP*α*CD, *L*Hist/*β*CD, *D*Hist/*α*CD, *D*Hist/HP*α*CD, and *D*Hist/*β*CD inclusion complexes

For the complexes formed between L-, D-Arg and CD, the recorded spectra show that the characteristic peaks of guanidyl group (about 1632 cm^{-1} and 1586 cm^{-1}), and the carboxylic and amino group at *α*-C (the frequencies of 1650 – 1658 cm^{-1}, 1402 – 1408 cm^{-1}, 1331 – 1334 cm^{-1}) are less shifted to lower wavenumbers in complexes formed with HP*α*CD and *α*CD than in those with *β*CD. For L- D- Arg/*β*CD complexes, the peak at about 1658 cm^{-1} is missing, suggesting that the guanidyl group is partially included in the cavity. Considering the physical dimensions of molecules, the Arg length of 1 nm exceeds the height of the CD torus of 0.8 nm, so despite a good fit inside the CD cavity of the hydrocarbon part of Arg, there is the possibility of partial inclusion of the terminal groups of Arg, (the amino groups of each part of molecule and carboxyl group, respectively), especially for HP*α*CD and *α*CD. These functional groups can be involved in bonds with the CD rims or not. The spectra of complexes formed between Hist and CDs show clearer differences in the complexation of different isomers than in Arg complexation. The spectra of complexes formed between Hist and CDs show some characteristic peaks of corresponding pure amino acids but are somewhat shifted to lower wavenumbers. For *D*Hist/CD complexes the frequencies of 1619 – 1620 cm^{-1}, 1588 cm^{-1}, 1454 cm^{-1}, 1411 cm^{-1}, 1361 cm^{-1}, and 1305 cm^{-1} are typical vibrations of a COO– group fragment, aliphatic hydrocarbon, and part of imidazole ring, suggesting the position near

to the CD rim and a partial inclusion in CD's cavity. In the recorded spectra of *L*Hist/CD complexes, the peaks of 1631 cm^{-1}, 1402 cm^{-1}, and 1332 cm^{-1} are shifted vibration frequencies of *L*Hist for COO^- and amino at *α*-C groups, suggesting that *L*Hist is involved in interactions with hydroxyl rims and strongly interact with the inner cavity of CD.

3.4. *Morphology studies*

The SEM images of the pure CD and the levo-forms of Arg and Hist have already been shown and described in some publications.[51,52] The SEM images (Fig. 11) show regular shapes for native compounds, especially for the pure HP*α*CD, which consists of very small particles with regular spherical shapes and large holes,[53,54] while the pure *α*CD and *β*CD consist of irregularly shaped crystals with smaller particles adhering to the surfaces of larger particles. For comparison, the L- and D-isomers of Arg and Hist were also characterized. L-, D-Arg appear as lamellar crystals with smooth surfaces and tend to form aggregates. SEM images of L-, D-Hist show well-developed tabular crystals, Figures 11c and 11d. The SEM images of the inclusion complexes indicate that the morphology of the two initial components is indistinguishable, suggesting the possibility of interaction between amino acid and the CD cavity.[55] For illustration, the SEM micrographs of the *L*Arg/*α*CD, *D*Arg/*α*CD, *L*Hist/HP*α*CD, *D*Hist/HP*α*CD, *L*Arg/HP*α*CD, *D*Arg/HP*α*CD, *L*Hist/*β*CD, and *D*Hist/*β*CD systems are shown in Figures 11e through 11l.

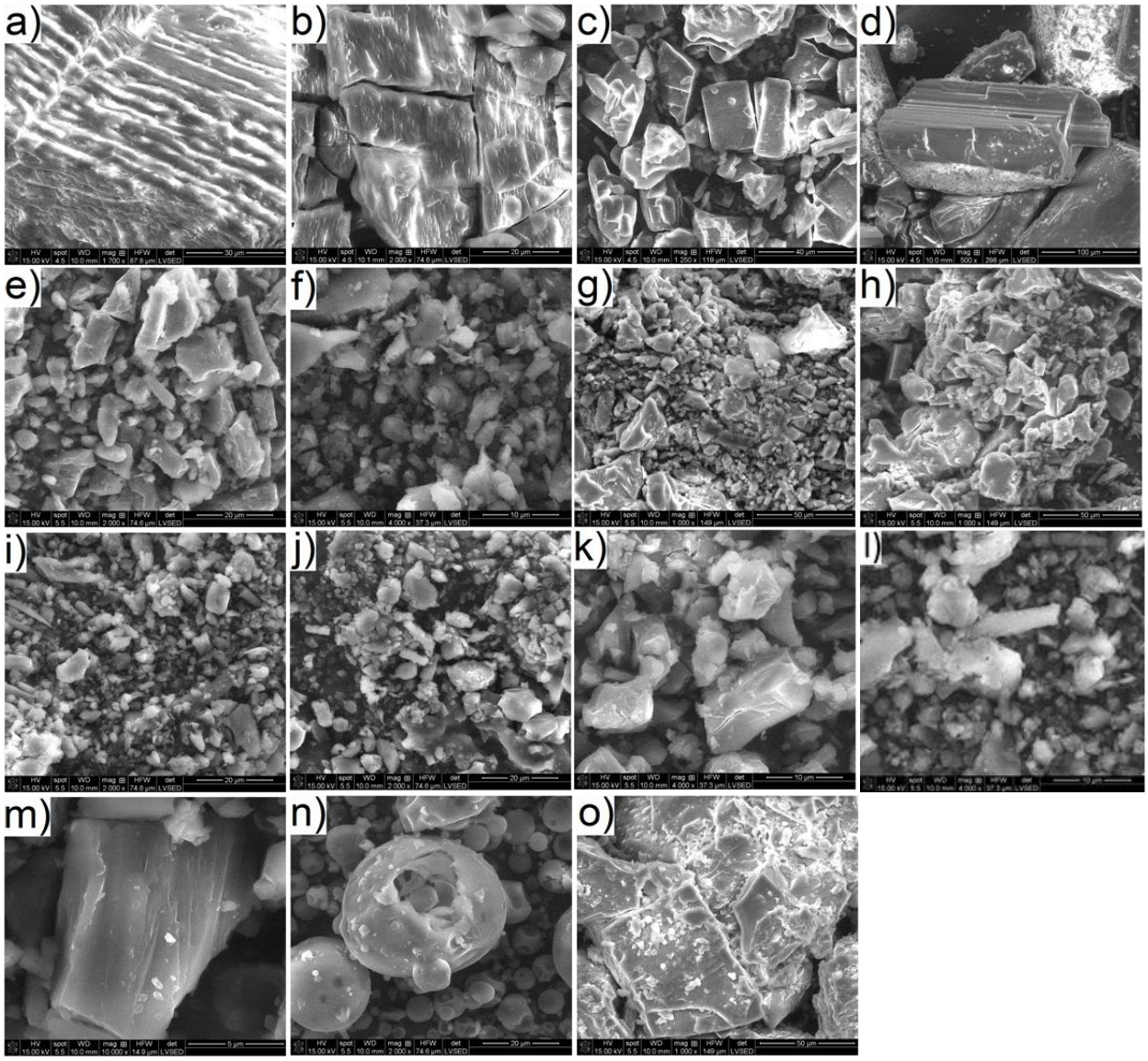

Fig. 11. SEM images of (a) - *L*Arg, (b) - *D*Arg, (c) - *L*Hist, (d) - *D*Hist, (e) - *L*Arg/*α*CD, (f) - *D*Arg/*α*CD, (g) - *L*Hist/HP*α*CD, (h) - *D*Hist/HP*α*CD, (i) - *L*Arg/HP*α*CD, (j) - *D*Arg/HP*α*CD, (k) - *L*Hist/*β*CD, (l) - *D*Hist/*β*CD, (m) - *α*CD, (n) - HP*α*CD, (o) - *β*CD at 25 ± 1 °C

In these systems, there is a marked difference in the shape and surface morphology of the crystals of the inclusion complex and the native CDs. However, the SEM method helps to prove the presence of a single component in the obtained products and provides some information about the efficiency of the complex formation.[37]

4. CONCLUSION

This work was concerned with the preparation of inclusion complexes of Arg and Hist stereoisomers with CDs using the co-precipitation method and the subsequent investigation of the complexes using TG/DSC, ATR, FTIR, and SEM analytical techniques. The correlation of the data resulting from the use of different characterization techniques provided evidence for the formation of inclusion complexes in the solid state by interaction of the amino acid with CD through hydrophobic and hydrogen bonding interactions. The relative thermal stability of the complexes is related to the CD type. The results show that complexes with HP*α*CD have the lowest thermal stability. This can be explained by the presence of the HP-substituents, which can interact with functional groups of amino acids that overhang from the HP*α*CD cavity, and therefore the inclusion complexes between HP*α*CD and L-, D-amino acids are destabilized by the HP-substituents on the rim of the CD. Considering the obtained data, the inclusion complexes formed between *α*CD, *β*CD, and L-, D-Arg were found to be more stable than the complexes formed between *α*CD, *β*CD, and L-, D-Hist. *L*Arg/*β*CD, *D*Arg/*β*CD, and *L*Hist/*β*CD complexes are more stable than the *D*Hist/*β*CD complex. Thus, it can be concluded that the size, symmetry, and functionalization of the truncated cone of the CD had a significant influence on the complexation interaction with Arg and Hist amino acids. However, the thermal stability of the complexes is also influenced by the amino acid structure and flexibility in interaction with CD. By correlation, it has been found that *β*CD has a preference to form the more stable complexes with levogyr isomers, *L*Arg and *L*Hist, respectively, and CDs could better discriminate between Hist isomers than between Arg isomers.

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REFERENCES

(1) Szejtli, J., Past, present and futute of cyclodextrin research. *Pure Appl. Chem.* **2004**, *10*, 1825–1845. https://doi.org/10.1351/pac200476101825

(2) Giordano, F.; Novak, C.; Moyano, J. R., Thermal analysis of cyclodextrins ant their inclusion compounds. *Thermochim. Acta* **2001**, *380*, 123–151. https://doi.org/10.1016/S0040-6031(01)00665-7

(3) Crini, G., Review: A history of cyclodextrin. *Chem. Rev.* **2014**, *114*(21), 10940–10975. https://doi.org/10.1021/cr500081p

(4) Araj, S. K.; Szeleszczuk, Ł., A review on cyclodextrins/estrogens inclusion complexes. *Int. J. Mol. Sci.* **2023**, *24*, 8780. https://doi.org/10.3390/ijms24108780

(5) Bajorunaite, E.; Cirkovas, A.; Radzevicius, K.; Larsen, K. L.; Sereikaite, J.; Bumelis, V. A., Anti-aggregatory effect of cyclodextrins in the refolding process of recombinant growth hormones from *Escherichia coli* inclusion bodies. *Int. J. Bio. Macromol.* **2009**, *44*(5), 428–434. https://doi.org/10.1016/j.ijbiomac.2009.03.005

(6) Ghosh, S.; Ghosh, C.; Nandi, S.; Bhattacharyya, K., Unfolding and refolding of a protein by cholesterol and cyclodextrin: A single molecule study. *Phys. Chem. Chem. Phys.* **2015**, *17*, 8017–8027. https://doi.org/10.1039/C5CP00385G

(7) Aachmann, F. L.; Otzen, D. E.; Larsen, K. L.; Wimmer, R., Structural background of cyclodextrin-protein interactions. *Protein. Eng.* **2003**, *16* (12), 905–912. https://doi.org/10.1093/protein/gzg137

(8) Rospiccio, M.; Arsiccio, A.; Winter, G.; Pisano, R., The role of cyclodextrins against interface-induced denaturation in pharmaceutical formulations: A molecular dynamics approach. *Mol. Pharm.* **2021**, *18*(6), 2322–2333. https://doi.org/10.1021/acs.molpharmaceut.1c00135

(9) Gingter, S.; Bezdushna. E.; Ritter, H., Chiral recognition of macromolecules with cyclodextrins: pH- and thermosensitive copolymers from *N*-isopropylacrylamide and *N*-acryloyl-D/L-phenylalanine and their inclusion complexes with cyclodextrins. *Beilstein J. Org. Chem.* **2011**, *7*, 204–209. https://doi.org/10.3762/bjoc.7.27

(10) Alexander, J.; Clark, J.; Brett, T.; Stezowski, J., Chiral discrimination in cyclodextrin complexes of amino acid derivatives: beta-cyclodextrin/*N*-acetyl-L-phenylalanine and *N*-acetyl-D-phenylalanine complexes. *PNAS*. **2002**, *99*(8), 5115–5120. https://doi.org/10.1073/pnas.072647599

(11) Liu, Y.; Bin, L.; Han, B.; Li, Y.; Chen, R., Enantioselective recognition of amino acids by beta-cyclodextrin 6-O-monophosphates. *J. Chem. Soc. Perk.* **1997**, *2*, 1275–1278. https://doi.org/10.1039/A700167C

(12) Liu, Y.; Zhang, Y.; Qi, A., Molecular recognition study on a supramolecular system. 10. Inclusion complexation of modified beta-cyclodextrins with amino acids: Enhanced enantioselectivity for L/D-leucine. *J. Org. Chem.* **1997**, *62*(6), 1826–1830. https://doi.org/10.1021/jo961625b

(13) Song, L. X.; Teng, C. F.; Yang, Y., Preparation and characterization of the solid inclusion compounds of *α*-, *β*-

cyclodextrin with phenylalanine (D-, L- and DL-Phe) and tryptophan (D-, L- and DL-Trp). *J. Incl. Phenom. Macrocycl. Chem.* **2006**, *54*(3), 221–232. https://doi.org/10.1007/s10847-005-7970-8

(14) Nagy, G.; Chouinard, C. D.; Attah, I. K.; Webb, I. K.; Garimella, S. V. B.; Ibrahim, Y. M.; Baker, E. S.; Smith R. D., Distinguishing enantiomeric amino acids with chiral cyclodextrin adducts and structures for lossless ion manipulations. *Electrophor*. **2018**, *39*(24), 3148–3155. https://doi.org/10.1002/elps.201800294

(15) Linde, G. A.; Laverde, A. Jr.; Vaz de Faria, E.; Colauto, N. B.; De Moraes F. F.; Zanin, G., Taste modification of amino acids and protein hydrolysate by α-cyclodextrin. *Food Res. Int*. **2009**, *42*(7), 814–818. https://doi.org/10.1016/j.foodres.2009.03.016

(16) Li J.; Wang H.; Yu D.; Zhang X, Stabilization effects of saccharides in protein formulation: A review of sucrose, trehalose, cyclodextrins and dextrans. *Eur. J. Pharm. Sci.* **2024**, *192*, 1066625. http://doi.org/10.1016/j.ejps.2023.106625

(17) Aachmann, F.; Larsen, K.; Wimmer, R., Interactions of cyclodextrins with aromatic amino acids: A basis for protein interactions. *J. Incl. Phenom. Macrocycl. Chem.* **2012**, *73*, 349–357. https://doi.org/10.1007/s10847-011-0071-y

(18) Cunniff, J. B.; Vouros, P., False positives and the detection of cyclodextrin inclusion complexes by electrospray mass spectrometry. *J. Incl. Phenom. Macrocycl. Chem.* **1995**, *6*(5), 437–447. https://doi.org/10.1016/1044-0305(95)00053-G

(19) Kundu, M.; Saha, S.; Roy, M. N., Evidences for complexations of *β*-cyclodextrin with some amino acids by ^{1}H NMR, surface tension, volumetric investigations and XRD. *J. Mol. Liq*. **2017**, *240*, 570–577. https://doi.org/10.1016/j.molliq.2017.05.123

(20) Neacşu, D. A.; Neacşu, A.; Contineanu, I.; Munteanu, G.; Tanasescu, S., Solid state study of the inclusion compounds of alpha-, beta-cyclodextrins with D-, L-tryptophan isomers. *Rev. Rou. Chim.* **2013**, *58*(11–12), 863–870. https://revroum.lew.ro/wp-content/uploads/2013/11/Art%2003.pdf

(21) Ekka, D.; Roy, M. N., Molecular interactions of *α*-amino acids insight into aqueous *β*-cyclodextrin systems. *Amino Acids*. **2013**, *45*(4), 755–777. https://doi.org/10.1007/s00726-013-1519-8

(22) Roy, M.; Roy, A.; Saha, S., Probing inclusion complexes of cyclodextrins with amino acids by physicochemical approach. *Carbohyd. Polym.* **2016**, *151*, 458–466. https://doi.org/10.1016/j.carbpol.2016.05.100

(23) Roy, M.; Ekka, D.; Saha, S.; Roy, M., Host-guest inclusion complexes of alpha and beta-cyclodextrins with alpha-amino acids. *RSC Advances*. **2014**, *4*, 42383–42390. https://doi.org/10.1039/C4RA07877B

(24) Wu, G; Meininger, C. J.; McNeal, C.; Bazer, F. W.; Rhoads, J. M., Role of L-arginine in nitric oxide synthesis and health in humans. *Adv. Exp. Med. Biol*. **2021**, *1332*, 167–187. https://doi.org/10.1007/978-3-030-74180-8_10

(25) Baynes, B. M.; Wang, D. I.; Trout, B. L., Role of arginine in the stabilization of proteins against aggregation. *Biochemistry*. **2005**, *44*(12), 4919–4925. https://doi.org/10.1021/bi047528r

(26) Arakawa, T.; Ejima, D.; Tsumoto, K.; Obeyama, N.; Tanaka, Y.; Kita, Y.; Timasheff, S. N., Suppression of protein interactions by arginine: a proposed mechanism of the arginine effects. *Biophys Chem*. **2007**, *127*(1–2), 1–8. https://doi.org/10.1016/j.bpc.2006.12.007

(27) Liao, S. M.; Du, Q. S.; Meng, J. Z.; Pang, Z. W.; Huang, R. B., The multiple roles of histidine in protein interactions. *Chem. Cent. J*. **2013**, *7(1)*, 44. https://doi.org/10.1186/1752-153X-7-44

(28) Holeček, M., Histidine in health and disease: metabolism, physiological importance, and use as a supplement. *Nutrients* **2020**, *12*(3), 848. https://doi.org/10.3390/nu12030848

(29) Saha, S.; Ray, T.; Basak, S.; Roy, N. M., NMR, surface tension and conductivity studies to determine the inclusion mechanism: thermodynamics of host–guest inclusion complexes of natural amino acids in aqueous cyclodextrins. *New J. Chem*. **2015**, *40*, 651–661. https://doi.org/10.1039/C5NJ02179K

(30) Esmaeilpour, D.; Shityakov, S.; Tamaddon, A. M.; Bordbar, A. K., Comparative chemical examination of inclusion complexes formed with β-cyclodextrin derivatives and basic amino acids. *Carbohydr Polym*. 2021, *262*, 117868. https://doi.org/10.1016/j.carbpol.2021.117868

(31) Benjamas, C.; Jaruporn, R., Inclusion complex formation of cyclodextrin with its guest and their applications. *Biol. Eng. Med*. **2016**, *2*(1), 1–6. https://doi.org/10.15761/BEM.1000108

(32) Lahiani-Skiba, M.; Boulet, Y.; Youm, I.; Bounoure F,; Verite P.; Arnaud, P.; Skiba, M., Interaction between hydrophilic drug and *α*-cyclodextrins: physico-chemical aspects. *J. Incl. Phenom. Macrocycl. Chem*. **2007**, *57*, 211–217. https://doi.org/10.1007/s10847-006-9194-y

(33) Shalaby, K. S.; Ismail, M. I.; Lamprecht, A., Cyclodextrin complex formation with water-soluble drugs: conclusions from isothermal titration calorimetry and molecular modeling. *AAPS Pharm. Sci. Tech*. **2021**, *22*(7), 232. https://doi.org/10.1208/s12249-021-02040-8

(34) Choi, K. E.; Chae, E.; Balupuri, A.; Yoon, H. R.; Kang, N. S., Topological water network analysis around amino acids. *Molecules* **2019**, *24*(14), 2653. https://doi.org/10.3390/molecules24142653

(35) Monera, O. D.; Sereda, T. J.; Zhou, N. E.; Kay, C. M.; Hodges, R. S., Relationship of sidechain hydrophobicity and alpha-helical propensity on the stability of the single-stranded amphipathic alpha-helix. *J. Pept. Sci*. **1995**, *1*(5), 319–329. https://doi.org/10.1002/psc.310010507

(36) Mohamed Ameen, H.; Kunsági-Máté, S.; Bognár, B.; Szente, L.; Poór, M.; Lemli, B., Thermodynamic characterization of the interaction between the antimicrobial drug sulfamethazine and two selected cyclodextrins. *Molecules*. **2019**, *24*(24), 4565. https://doi.org/10.3390/molecules24244565

(37) Sgarlata, C.; Mugridge, J. S.; Pluth, M. D.; Zito, V.; Arena, G.; Raymond, K. N., Different and often opposing forces drive the encapsulation and multiple exterior binding of charged guests to a M_4 L_6 supramolecular vessel in water. *Chemistry* **2017**, *23*(66), 16813–16818. https://doi.org/10.1002/chem.201703202

(38) Loftsson, T.; Sigurdsson, H. H.; Jansook, P., Anomalous properties of cyclodextrins and their complexes in aqueous solutions. *Materials (Basel)* **2023**, *16*(6), 2223. https://doi.org/10.3390/ma16062223

(39) D'Aria, F.; Pagano, B.; Giancola, C., Thermodynamic properties of hydroxypropyl-*β*-cyclodextrin/guest interaction: a survey of recent studies. *J. Therm. Anal. Calorim.* **2022**, *47*, 4889–4897. https://doi.org/10.1007/s10973-021-10958-1

(40) George, S. J.; Vasudevan, D. T., Studies on the preparation, characterization, and solubility of 2-HP-*β*-cyclodextrin-meclizine HCl inclusion complexes. *J. Young Pharm.* **2012**, *4*(4), 220–227. https://doi.org/10.4103/0975-1483.104365

(41) Menezes, P. P.; Serafini, M. R.; Quintans-Junior, L. J.; Silva, G. F.; Oliveira, J. F.; Carvalho, F. M. S.; Souza, J. C. C.; Matos, J. R.; Alves, P. B.; Matos, I. L.; Hadaruga, D. I.; Araujo, A. A. S., Inclusion complex of (2)-linalool and b-cyclodextrin. *J. Therm. Anal. Calorim.* **2014**, *115*(3), 2429–2437. https://doi.org/10.1007/s10973-013-3367-x

(42) Teixeira, L. R.; Sinisterra, R. D.; Vieira R. P.; Scarlatelli-Lima, A.; Moraes, M. F. D.; Doretto, M. C.; Denadai, A. M.; Beraldo, H., An inclusion compound of the anticonvulsant sodium valproate into a-cyclodextrin: physico-chemical characterization. *J. Incl. Phenom. Macrocyclic Chem*. **2006**, *54*(1), 133–138. https://doi.org/10.1007/s10847-005-5817-y

(43) Song, L. X.; Teng, C. F.; Xu, P.; Wang, H. M.; Zhang, Z. Q.; Liu, Q. Q., Thermal decomposition behaviors of b-cyclodextrin, its inclusion complexes of alkyl amines, and complexed b-cyclodextrin at different heating rates. J. *Incl. Phenom. Macrocycl. Chem*. **2008**, *60*(3), 223–233. https://doi.org/10.1007/s10847-007-9369-1

(44) Hou, Y.; Li, S.; Sun, T.; Yang, J.; Xing, P.; Liu, W.; Hao, A., Organogels based on b-cyclodextrin system with molecular recognition property. *J. Incl. Phenom. Macrocycl. Chem.* **2014**, *80*(3), 217–224. https://doi.org/10.1007/s10847-013-0379-x

(45) Rocha, B. A.; Rodrigues, M. R.; Bueno, P. C. P.; de Mello Costa-Machado, A. R.; De Oliveira Lima Leite Vaz, M. M.; Nascimento, A. P.; Barud, H. S.; Berretta-Silva, A. A., Preparation and thermal characterization of inclusion complex of Brazilian green propolis and hydroxypropyl-b-cyclodextrin. *J. Therm. Anal. Calorim*. **2012**, *108*(1), 87–94. https://doi.org/10.1007/s10973-011-1713-4

(46) Orgoványi, J.; Oláh, E.; H-Otta, K.; Fenyvesi, E., Dissolution properties of cypermethrin/cyclodextrin complexes. *J Incl. Phenom. Macrocycl. Chem*. **2009**, *63*(1), 53–59. https://doi.org/10.1007/s10847-008-9488-3

(47) Lakkakula, J.; Krause R. W. M.; Ndinteh, T. D.; Vijaylakshmi, S. P.; Raichur, M. A., Detailed investigation of a γ-cyclodextrin inclusion complex with L-thyroxine for improved pharmaceutical formulations. *J. Incl. Phenom. Macrocycl. Chem*. **2012**, *74*(1), 397–405. https://doi.org/10.1007/s10847-012-0133-9

(48) Nicolescu, C.; Arama, C.; Monciu, C. M., Preparation and characterization of inclusion complexes between repaglidine and *β*-cyclodextrin, 2-hydroxypropyl-*β*-cyclodextrin and randomyl methtlated *β*-cyclodextrin. *Farmacia* **2010**, *58*(1), 78–88. https://farmaciajournal.com/arhiva/20101/issue12010art09.pdf

(49) Sbora, R.; Budura, E. A.; Nitulescu, G. M.; Balaci, T.; Lupuleasa, D., Preparation and characterization of inclusion complexes formed by avobenzone with *β*-cyclodextrin, hydroxypropyl-*β*-cyclodextrin and hydroxypropyl-*α*-cyclodextrin. *Farmacia* **2015**, *63*(4), 548–555. https://farmaciajournal.com/wp-content/uploads/2015-04-art-13-Sbora_Budura_548-555.pdf

(50) Ikuta, N.; Tanaka, A.; Otsubo, A.; Ogawa, N.; Yamamoto, H.; Mizukami, T.; Arai, S.; Okuno, M.; Terao, K.; Matsugo, S., Spectroscopic studies of R(+)-*α*-lipoic acid–cyclodextrin complexes. *Int. J. Mol*. Sci. **2014**, *15*(11), 20469–20485. https://doi.org/10.3390/ijms151120469

(51) Menahem, T.; Mastai, Y., Chiral soluble polymers and microspheres for enantioselective crystallization. J. *Polym. Sci. A Polym. Chem*. **2006**, *44*(9), 3009–3017. https://doi.org/10.1002/pola.21376

(52) Lakio, S.; Morton, D. A. V.; Ralph, A. P.; Lambert, P., Optimizing aerosolization of a high-dose L-arginine powder for pulmonary delivery. *Asian J. Pharm. Sci.* **2015**, *10*(6), 528–540. https://doi.org/10.1016/j.ajps.2015.08.001

(53) Mohit, V.; Harshal, G.; Neha, D.; Vilasrao, K.; Rajashree, H., A comparative study of complexation methods for cefdinir-hydroxypropyl-*β*-cyclodextrin system. *J. Incl. Phenom. Macrocycl. Chem*. **2011**, *71*(1), 57–66. https://doi.org/10.1007/s10847-010-9901-6

(54) Serafini, M. R.; Menezes, P. P.; Costa, L. P.; Lima, C. M.; Quintans Jr., L. J.; Cardoso, J. C.; Matos, J. R.; Soares-Sobrinho, J. L.; Grangeiro Jr., S.; Nunes, P. S.; Bonjadim, L. R.; Araújo, A. A. S., Interaction of p-cymene with b-cyclodextrin. *J. Therm. Anal. Calorim*. **2012**, *109*(2), 951–955. https://doi.org/10.1007/s10973-011-1736-x

(55) Li, S.; Yue, J.; Zhou, W.; Li, L., An investigation into the preparation, characterization and antioxidant activity of puerarin/cyclodextrin inclusion complexes. J. *Incl. Phenom. Macrocycl. Chem*. **2015**, *82*(3), 453–460. https://doi.org/10.1007/s10847-015-0516-9