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CHARACTERIZATION OF INCLUSION COMPLEXES FORMED BY 4-METHYLBENZYLIDENE CAMPHOR WITH β – CYCLODEXTRIN, HYDROXYPROPYL – β – CYCLODEXTRIN AND HYDROXYPROPYL – α – CYCLODEXTRIN IN 1:2 MOLAR RATIO

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ABSTRACT. The aim of the present study was to confirm the formation of inclusion complexes between 4-Methylbenzylidene camphor and beta-cyclodextrin, hydroxypropyl-beta-cyclodextrin and hydroxypropyl-alfa-cyclodextrin, using lyophilization technique. The inclusion complexes were subjected to physicochemical characterizations in comparison with the simple physical mixtures prepared in the same 1:2 (4-Methylbenzylidene camphor : cyclodextrin) molar ratio. The complexes were evaluated by the scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FT-IR). The inclusion complexes between 4-Methylbenzylidene camphor and beta-cyclodextrins, in 1:2 molar ratio, found better in all the studied parameters in comparison with the complexes prepared with alfa-cyclodextrin, probably due to the 4-Methylbenzylidene camphor molecule size which fits better in beta-cyclodextrins than in alfa-cyclodextrins cavity.
KEYWORDS: 4-Methylbenzylidene camphor, beta-cyclodextrin, hydroxypropyl-beta-cyclodextrin, hydroxypropyl-alfa-cyclodextrin, inclusion complex, lyophilization.

INTRODUCTION

4-Methylbenzylidene camphor (4-MBC – fig.1) is an important organic UVB filter (290 – 320 nm), widely used in sunscreen and cosmetic products. Although 4-MBC has been regarded as rather photostable, recent studies have demonstrated that this sunscreen agent undergoes marked degradation under sun-light exposure. Therefore, new systems with enhanced 4-MBC photostability are required. Various methods have been developed to reduce photodecomposition of 4-Methylbenzylidene camphor: encapsulation in liposomes, loading in solid nanoparticles, or adding a photostabilizer into the formulation. Complexation of 4-MBC with cyclodextrins offers the possibility to enhance its stability, and decrease its degradation when exposed to light. (Scalia et al., 2007)

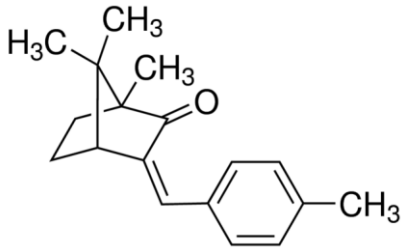


Figure 1 - The 4-Methylbenzylidene camphor's structure

Cyclodextrins (CDs) are cyclic oligosaccharides produced by enzymatic degradation of the starch, by the action of cyclodextrin-glycosyl-transferase. They have a hydrophilic character at the external surface and a lipophilic one at the internal cavity level. CDs act as host molecules to form inclusion complexes with a wide variety of guest molecules. (Loftsson, 1996). Their internal hydrophobic cavity allows the inclusion of lipophilic entities, resulting in enhanced water solubility, stability and bioavailability of various drugs. (Budura et al., 2011)

In the present study, we prepared by lyophilization method, investigated and confirmed the formation of three inclusion complex between 4-MBC and 2- beta-cyclodextrin (β-CD), hydroxypropyl-beta-cyclodextrin (HP-β-CD), hydroxypropyl-alfa-cyclodextrin (HP-α-CD). To characterize the properties

of the complexes in solid state we used scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FT-IR) analyses.

MATERIALS AND METHODS

Materials

4-MBC and CDs were purchased from Sigma -Aldrich Chemie GmbH, Germany. The methanol and distilled water were of analytical grade. For the weighing of the substances we used a Mettler Toledo AT261 balance (with 0.01 mg sensitivity).

Methods

Preparation of the complexes

In order to obtain the inclusion complexes of 4-MBC with β-CD, HP-β-CD, HP-α-CD we used the lyophilization method of complexation in solution, and to compare the results of characterisation tests of the complexes we prepared simple physical mixture in the same ratio (1:2).

Preparation of physical mixture between 4-MBC and β-CD, HP-β-CD, HP-α-CD

The substances weighted according to the molar ratio of 1:2 were physically mixed for 15 minutes, at the room temperature, in a mortar, until a homogeneous powder was obtained.

0.25 mmol of each CD were dissolved separately in 100 mL of distilled water. 15 mL of methanolic solution containing 0.125 mmol of 4-MBC was added stepwise to each aqueous solution of CD. The suspension was stirred with Heidolph MR 3001K magnetic stirrer, for 6 h, at 750 rpm, at room temperature, then the final suspension was lyophilized at -60°C, for 12 h, using the Christ ALPHA 1 – 2, B Braun Biotech International, Germany liophilizer. (Budura et al., 2011)

Both inclusion complexes obtained, and the physical mixtures were evaluated by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FT-IR) analyses.

Scanning electron microscopy (SEM)

The morphology of samples was determined using the scanning electron microscope, VEGA II model, produced by TESCAN, Czech Republic. The images were registered using the following parameters: accelerating voltage of 30 kV, at a working distance of 9.9 mm, with a beam current of 60 μA, a "probe current", PC, between 1:8 - 1:12, current absorbed up to ≈ 100pA, and secondary electrons detector. The physical rezolution on the samples surface is about half of the electrons spot size, which under these conditions is of maximum 50 nm. The images were registered at magnifications between 100x-4000x, with a fixed digitization rezolution of 1024 pixels x 1024 pixels. Although, not all samples have an enough conductivity, insted metallization, we choosed the working version with low vacuum in the samples room. So, at a presure in the samples room of 10 Pa the electrostatic charge is well enough eliminated. Samples were prepared by taking over by adhesion on a support covered with a graphite double adhesive conducting tape. The excess of the materials were eliminated by blowing with a compressed nitrogen gun, of chromatographic purity. All samples were analyzed in duplicate.

Differential scanning calorimetry (DSC)

DSC analyses of the samples were performed on SETARAM DSC 131EVO, equipped with a cooler system based on liquid nitrogen (95°C/min), a data detection system Platinum™ Software, and a DSC Standard Cell RC module. Samples of approximately 5 mg were weighed and sealed in aluminium pans. The samples were heated with a rate of 10°C/min, in an inert nitrogen atmosphere with a flow rate of 50.0 mL/min, in a temperature range of 30°C -210°C. As reference an empty sealed aluminium pan was used. Temperature and enthalpy ere calibrated with the standard material indium (99.98%, melting point: 156.65°C). The samples were analyzed in duplicate.

Fourier-transform infrared spectroscopy (FT-IR)

The Fourier Transform Infrared spectra were recorded using a JASCO FT/IR-4200 spectrometer with an ATR PRO450-S accessory, on a spectral range of 4000-400 cm^{-1} and a resolution of 4 cm^{-1}. The compounds were analysed in duplicate. The spectra are presented in transmittance percentages (T%) over wavelength (cm^{-1}).

RESULTS AND DISCUSSION

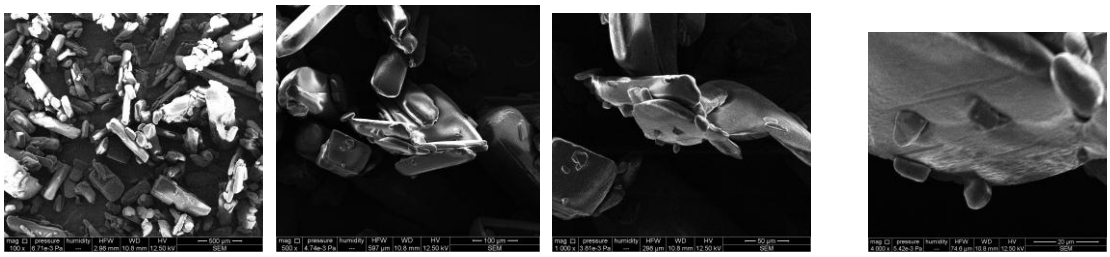
Organoleptic evaluation of the complexes

The physical mixtures obtained by mixing 4-MBC with CDs were white, crystalline powders, odorless, with a bitter taste. The complex prepared by lyophilization we obtained a very fine and smooth white powder, odorless and bitter tasted.

Scanning electron microscopy (SEM)

The SEM images of 4-MBC, β-CD, HP-β-CD, HP-α-CD, their physical mixtures, their lyophilization complex are shown in figures 2 to 11. The images were captured with a magnification between 100x and 4000x, so the uniformity on a large quantity of powder and also the details of the samples, can be seen.

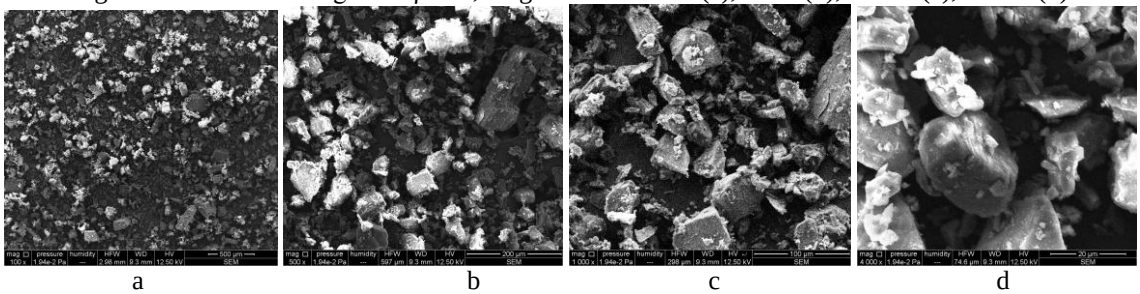
Figure 2 – The SEM images for 4-MBC, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



a b c d

4-MBC is characterized by the presence of hard crystalline particles, with various sizes, sizeable, between 5 and 30 μm.

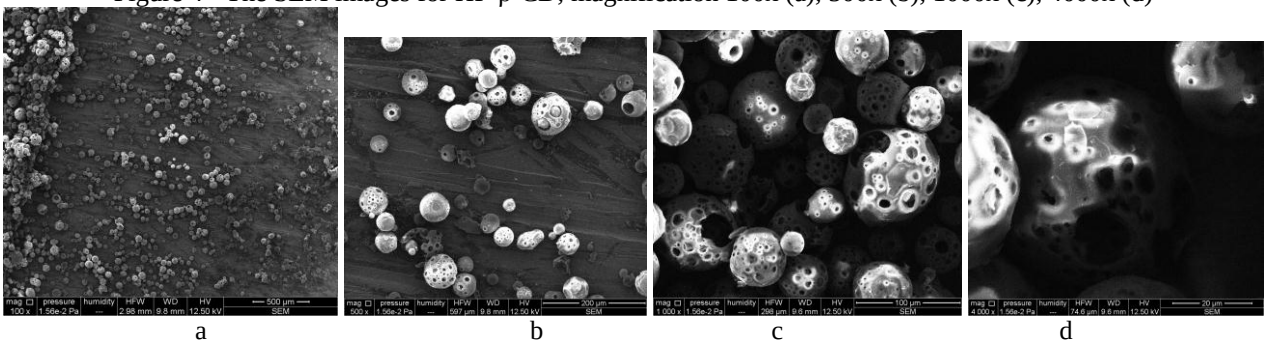
Figure 3 - The SEM images for β-CD, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



a b c d

β-CD has also crystalline particles, with various shapes and sizes between 5 and 20 μm.

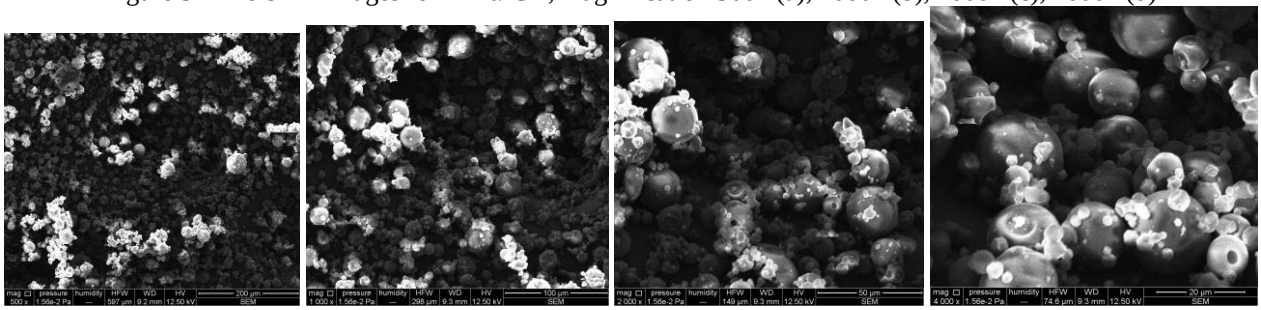
Figure 4 - The SEM images for HP-β-CD, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



a b c d

HP-β-CD has round particles, and an amorphous character.

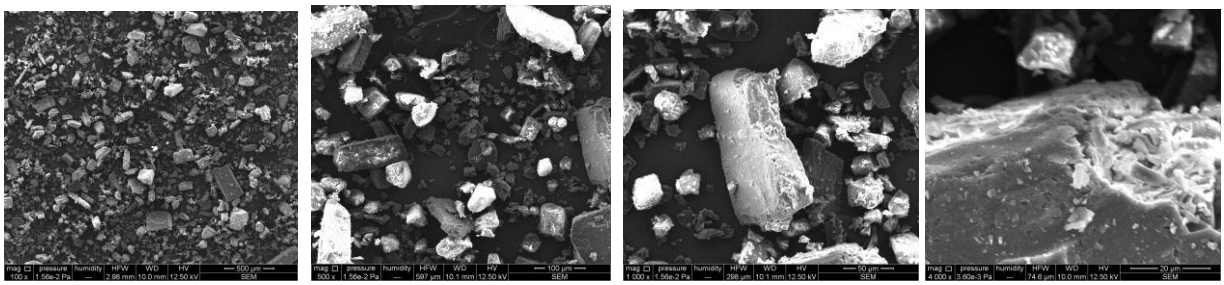
Figure 5 - The SEM images for HP-α-CD, magnification 500x (a), 1000x (b), 2000x (c), 4000x (d)



a b c d

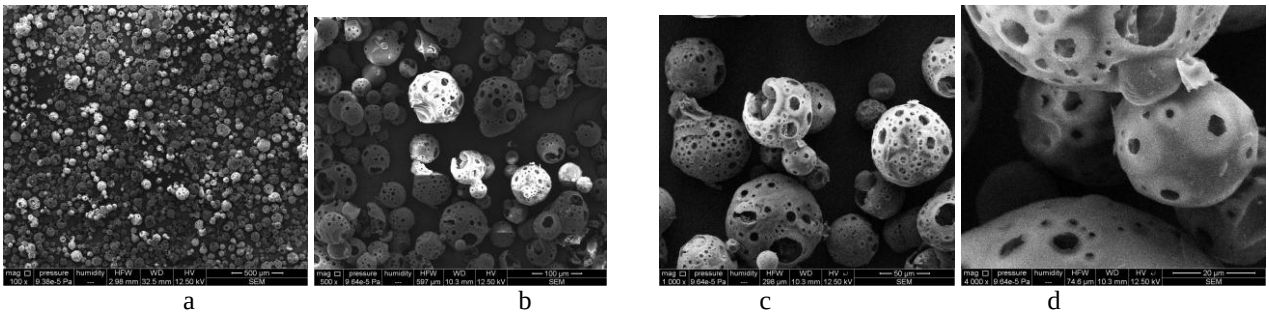
HP-α-CD is an amorphous powder, composed of round particles, with a diameter between 2 and 20 μm.

Figure 6 - The SEM images for 4-MBC-β-CD physical mixture, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



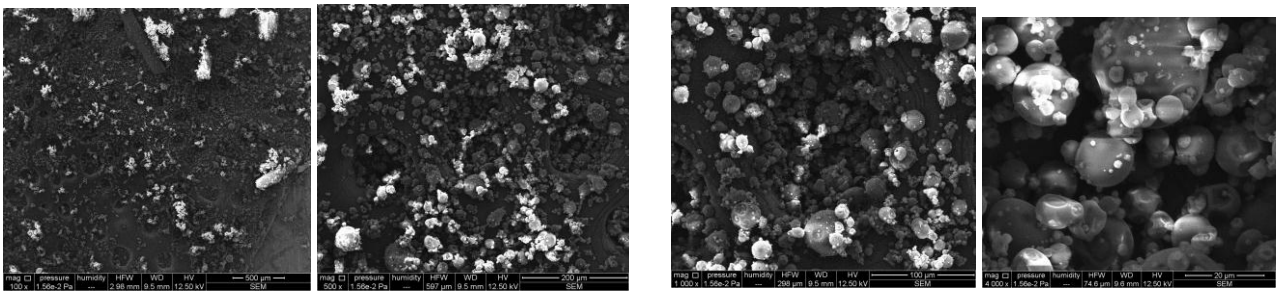
a b c d

Figure 7 - The SEM images for 4-MBC-HP-β-CD physical mixture, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



a b c d

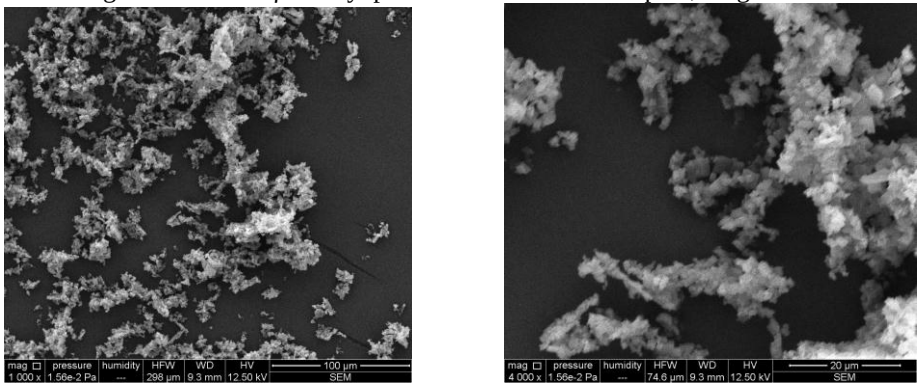
Figure 8 - The SEM images for 4-MBC-HP-α-CD physical mixture, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



a b c d

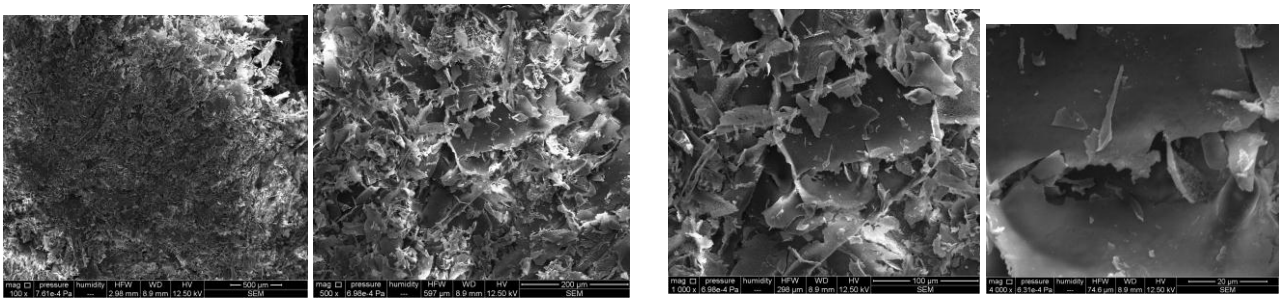
The SEM images for the 4-MBC- CDs physical mixtures shown the characteristics of 4-MBC crystals mixed with the crystalline or the amorphous particles of complexation agent, but the crystals of the active ingredient and the particles of CD were seen adhering to their surface, this confirming the affinity between substrates.

Figure 9 - The SEM images for 4-MBC-β-CD lyophilization inclusion complex, magnification 1000x (a), 4000x (b)



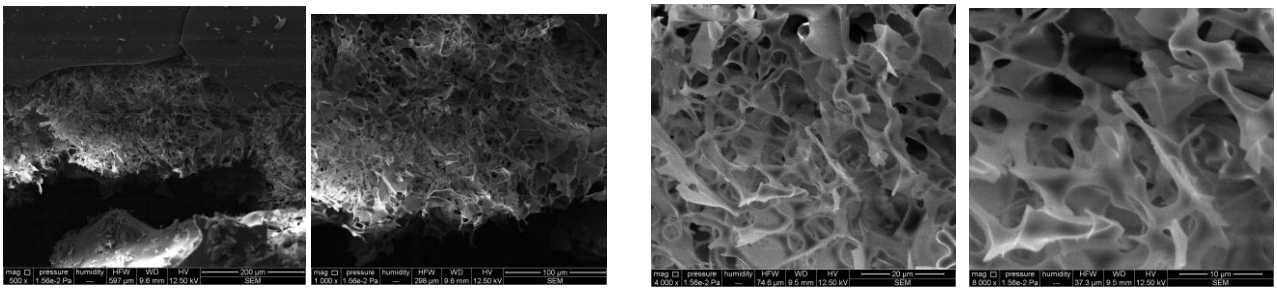
a b

Figure 10 - The SEM images for 4-MBC-HP-β-CD lyophilization inclusion complex, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



a b c d

Figure 11 - The SEM images for 4-MBC-HP-α-CD lyophilization inclusion complex, magnification 100x (a), 500x (b), 1000x (c), 4000x (d)



a b c d

In contrast with the simple physical mixtures, the lyophilization complexes shown a drastic change in the morphology and shape of the particles. In the lyophilization case, it was no longer possible to distinguish the two components, 4-MBC and CDs, revealing a strong interaction between the drug and CDs in these systems.

The SEM images of lyophilization inclusion complexes claerly shown the characteristic morphology as a small sized particles forming homogenous aggregates, indicating the existence of an amorphous product with presence of single component in the complex, this suggesting maximum complexation.

Differential scanning calorimetry (DSC)

The DSC thermogram of 4-MBC (figure 12) shown an endothermic peak at 69.37°C, corresponding to its melting point. The shape of the plot reveals the characteristics of a crystalline substance.

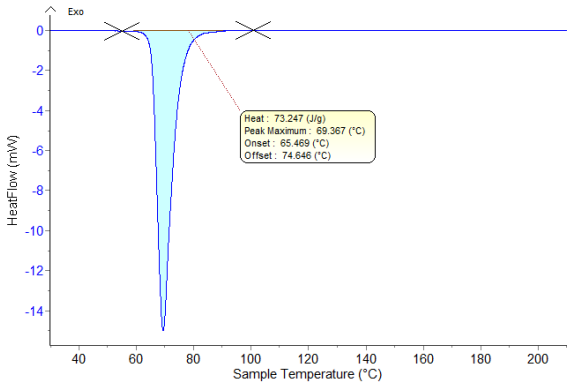


Figure 12 – The DSC thermogram of 4-MBC

The DSC thermograms of β-CD, HP-β-CD, and HP-α-CD showed one endothermic peak at temperature values corresponding to their melting point: 133.95°C for β-CD (figure 13), 133.95°C for HP-β-CD (figure 14), and 109.28°C for HP-α-CD (figure 15). The shape of the plot being characteristic for crystalline powder in case of β-CD, and for amorphous substances in case of HP-β-CD and HP-α-CD.

Figure 13 – The DSC thermogram of β-CD

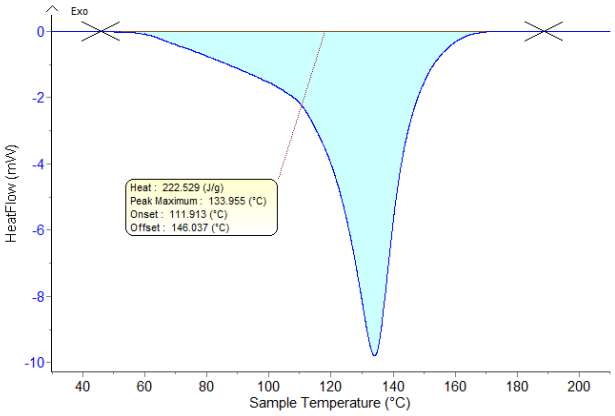


Figure 14 – The DSC thermogram of HP-β-CD

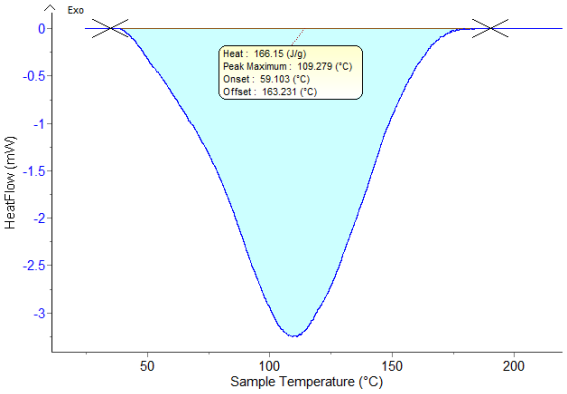
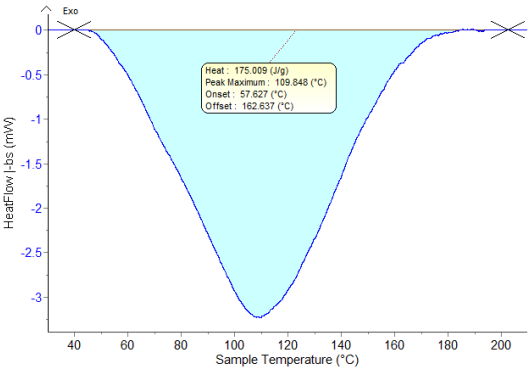


Figure 15 – The DSC thermogram of HP-α-CD



The DSC thermograms of 4-MBC-CDs physical mixtures (figures 16, 17, 18) shown, each of them, two endothermic peaks, one characteristic for 4-MBC at around 70°C, but with a low intensity, with a decreased endothermic effect, and the other one characteristic for the CDs in the mixture. This result proves that the two substrates interacted, but the complexation phenomenon didn't occur.

Figure 16 – The DSC thermogram of 4-MBC-β-CD physical mixture

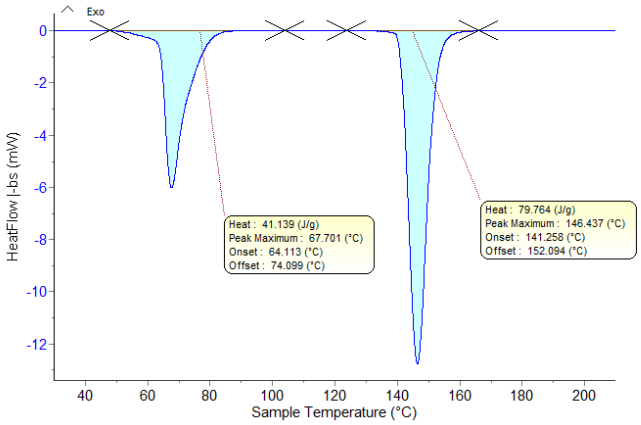


Figure 17 – The DSC thermogram of 4-MBC-HP-β-CD physical mixture

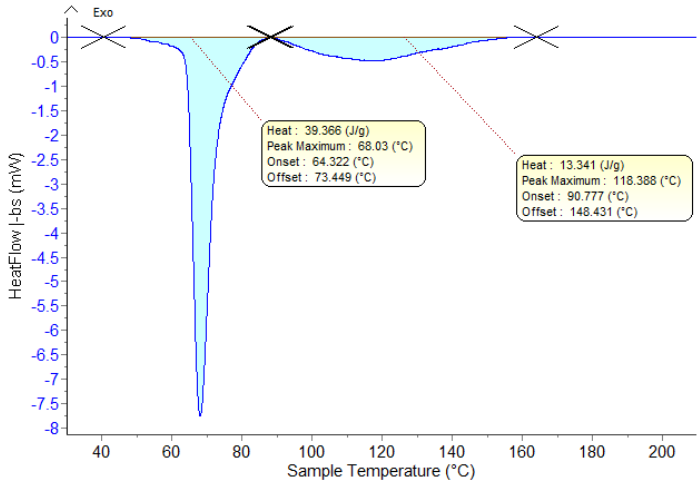
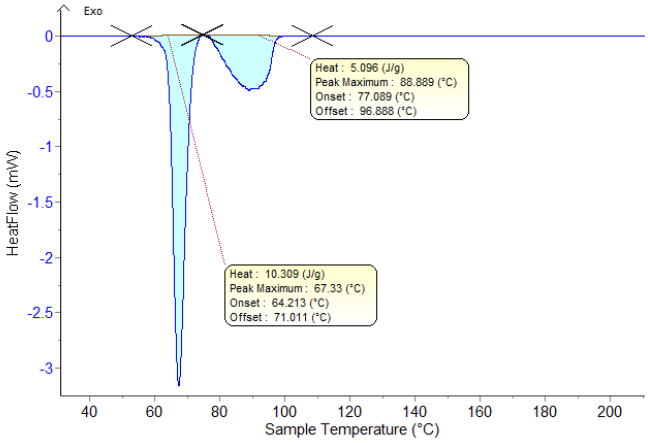


Figure 18 – The DSC thermogram of 4-MBC -HP-α-CD physical mixture



The DSC thermograms of 4-MBC-CDs lyophilization inclusion complexes (figures 19, 20, 21) showed only one endothermic prominent peak: at 116.58°C with an endothermic effect of 1.79 J/g for the 4-MBC-β-CD complex, at 96.09°C with a large endothermic effect of 153.93 J/g for the 4-MBC-HP-β-CD complex, and for the 4-MBC-HP-α-CD complex two peaks were registered at 111.20°C with a large endothermic effect of 137.15 J/g, and the other one at 63.87°C with a small endothermic effect of 5.49 J/g. This last peak is characteristic for 4-MBC, but has a much lower intensity. For the 4-MBC -β-CD and 4-MBC -HP-β-CD complexes remarkable is the complete disappearance of endothermic peak corresponding to 4-MBC, and the DSC curves have the characteristic shape of the amorphous substances, by all these proving the total forming of the inclusion complex. In the meantime it is noticed that in case of 4-MBC -HP-α-CD complex the complexation of 4-MBC appeared, but was not total.

Figure 19 – The DSC thermogram of 4-MBC -β-CD lyophilization inclusion complex

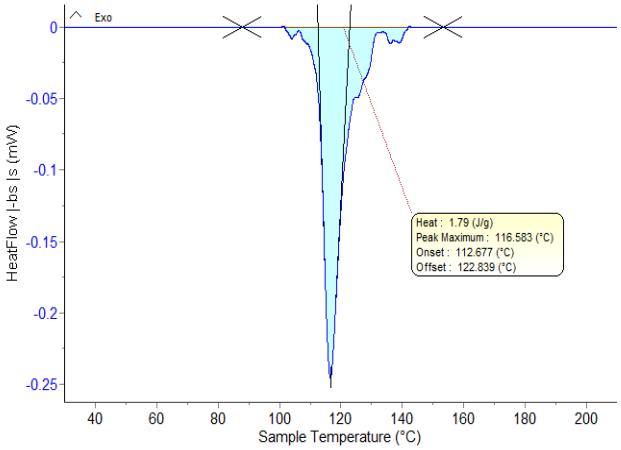


Figure 20 – The DSC thermogram of 4-MBC -HP-β-CD lyophilization inclusion complex

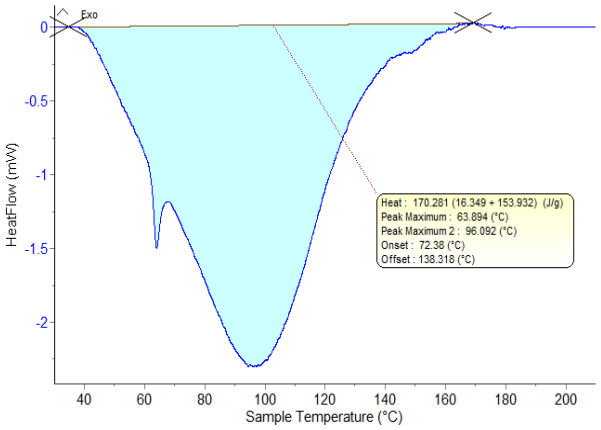
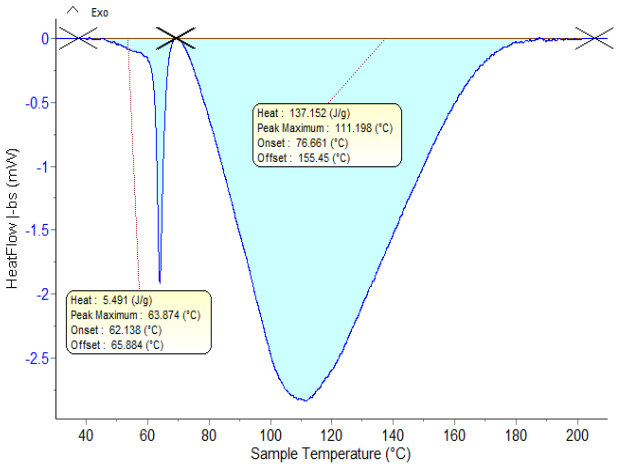


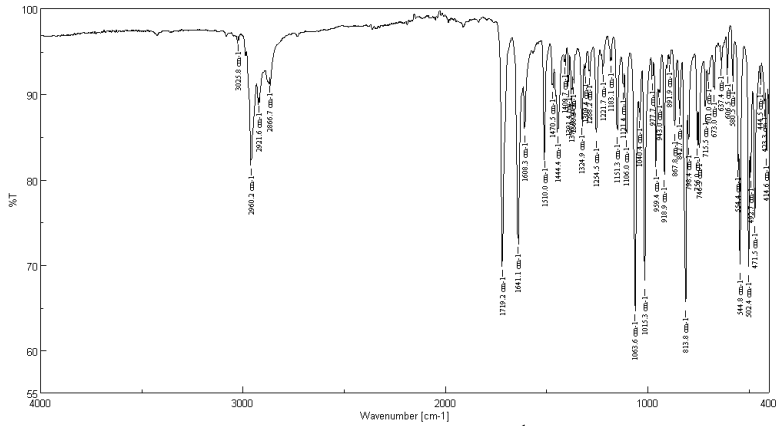
Figure 21 – The DSC thermogram of 4-MBC -HP-α-CD lyophilization inclusion complex



Fourier-transform infrared spectroscopy (FT-IR)

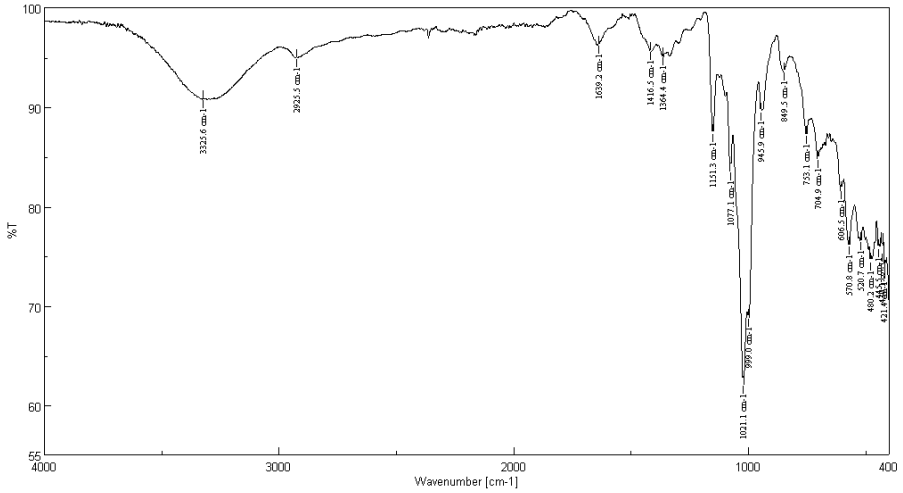
The FT-IR spectra of 4-MBC (figure 22) showed the presence of the an intense band at 1719 cm^{-1} characteristic for the carbonyl group, an intense band at 1641 cm^{-1} produced by C=C bond's stretch, an intense band at 1064 cm^{-1} produced by =C-H bond's deformation vibration, and a weak band at 3026 cm^{-1}, due to the =C-H stretching vibration.

Figure 22 – The FT-IR spectra of 4-MBC



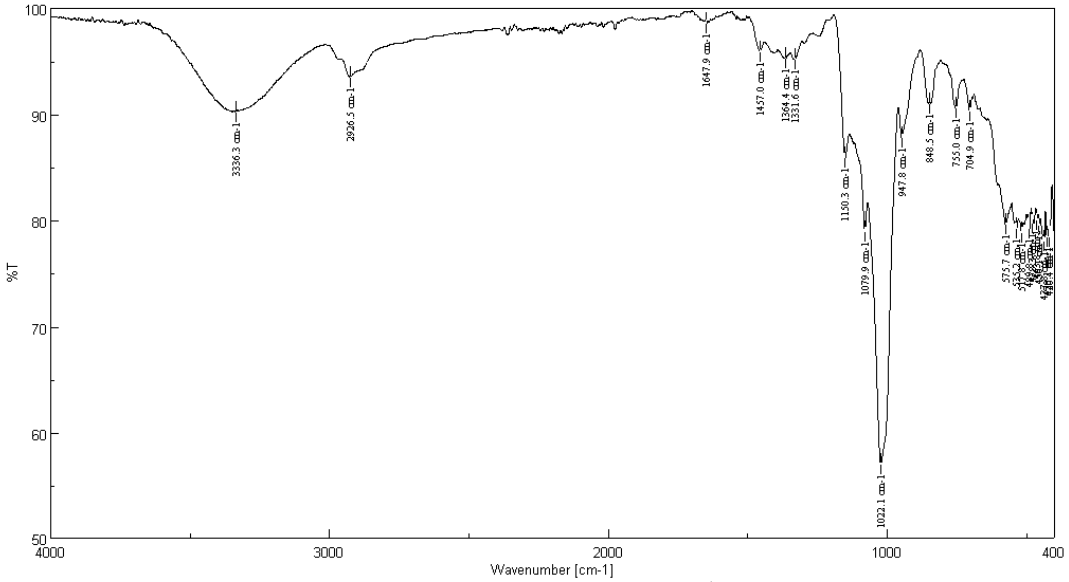
The FT-IR spectra of β-CD (figure 23) showed a large band at 3327 cm^{-1} produces by the O–H stretching vibration, and an intense band at 1021 cm^{-1} produced by the C–O bond's vibration.

Figure 23 – The FT-IR spectra of β-CD



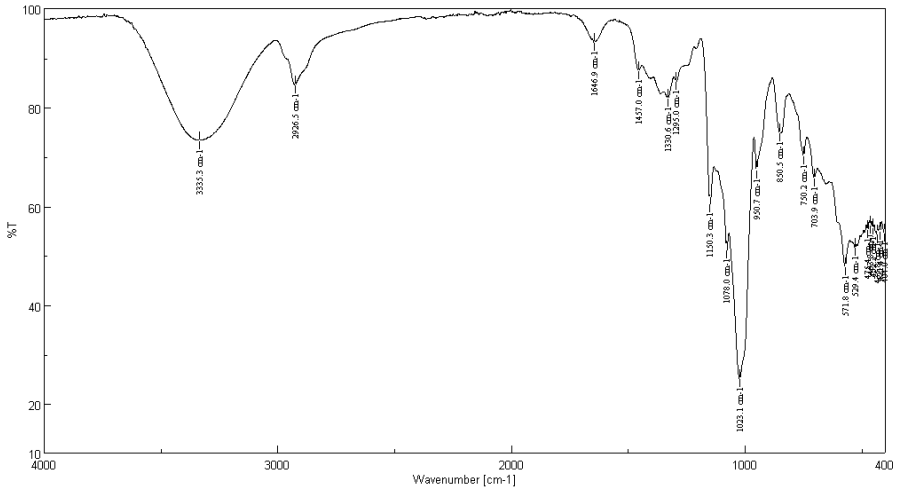
The FT-IR spectra of HP-β-CD (figure 24) is very similar to that of HP-α-CD, presenting a large band at 3336 cm^{-1}, due to the O–H stretching vibrations, and another strong band at 1022 cm^{-1} produced by the C–O bond's vibration. The alkyl region is pointed out by a band with a maximum at 2927 cm^{-1} wavelength.

Figure 24 – The FT-IR spectra of HP-β-CD



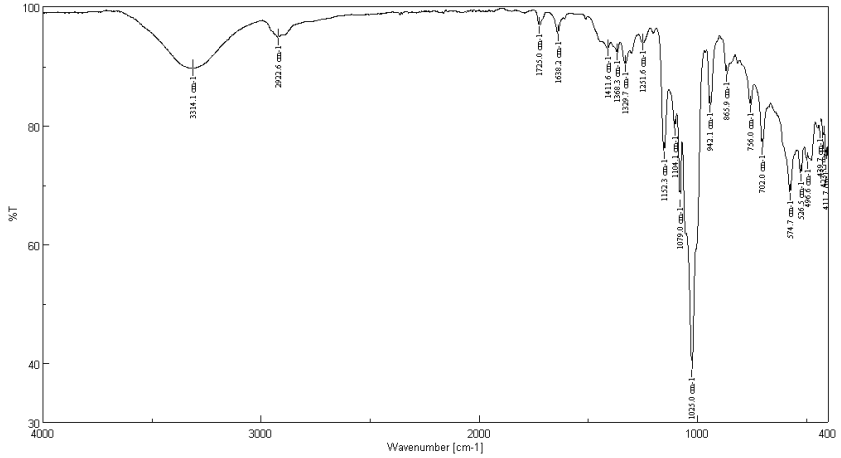
The FT-IR spectra of HP-α-CD (figure 25) also has a large band at 3335 cm^{-1} produced by the O–H stretching vibration, and another strong band at 1023 cm^{-1} corresponding to the C–O bond's vibration. The alkyl part is pointed out by a band with a maximum at 2927 cm^{-1} wavelength.

Figure 25 – The FT-IR spectra of HP-α-CD



The FT-IR spectra of 4-MBC-β-CD inclusion complex (figure 26) presents major spectral changes in comparison with the two separate substances spectra and with their physical mixture. The wavelengths in β-CD are suffering small changes, so the O–H stretching vibration decreases from 3327 cm^{-1} in β-CD to a value of 3314 cm^{-1}, probably due to the new formed hydrogen intermolecular bonds. Instead, the characteristic spectral portion for 4-MBC significantly decreases in intensity, mainly in the absorbtion of carbonyl bond band from 1725 cm^{-1}, and of C=C bond at 1638 cm^{-1}.

Figure 26 – The FT-IR spectra of 4-MBC-β-CD lyophilization inclusion complex



The FT-IR spectra of 4-MBC-HP-β-CD inclusion complex (figure 27) presents a high resemblance with the one of 4-MBC-HP-α-CD inclusion complex (figure 28), and with de CD alone, the spectral part characteristic for 4-MBC approximately nullified.

Figure 27 – The FT-IR spectra of 4-MBC- HP-β-CD lyophilization inclusion complex

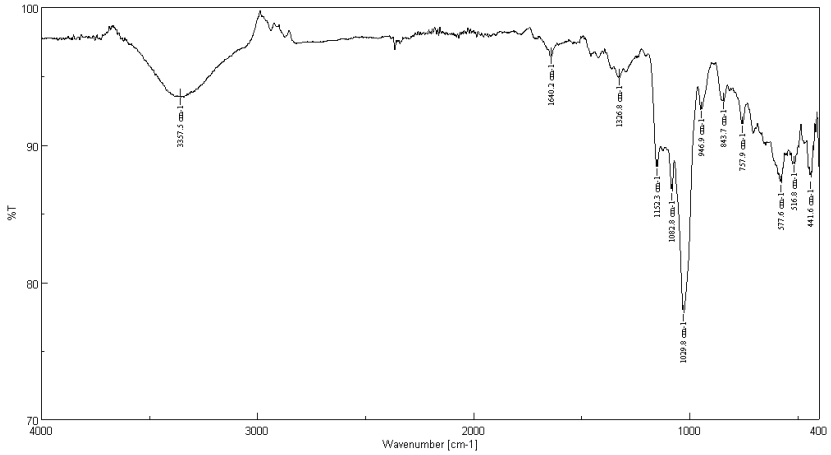
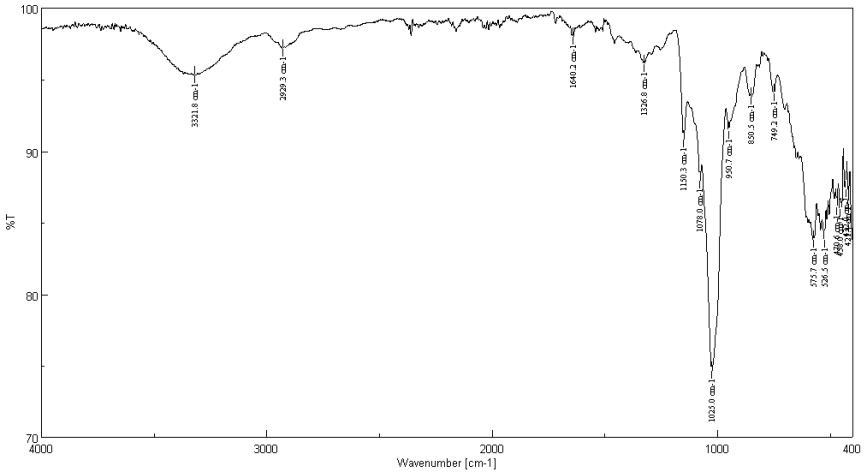


Figure 28 – The FT-IR spectra of 4-MBC-HP-α-CD lyophilization inclusion complex



CONCLUSIONS

The studied inclusion complexes between 4-MBC and β-CD, HP-β-CD, and HP-α-CD were prepared in a molar ratio of 1:2, by lyophilization method, when amorphous powders were obtain. In order to compare the results of the complexes analyses, we prepared three physical mixture between 4-MBC and the corresponding CDs, also in a molar ratio of 1:2, when a crystalline powder was obtained.

By evaluating the morphology of the inclusion complexes, using SEM technique, in comparison with the initial substances and their physical mixture, we noticed a remarkable change on the shape, sizes, and structure of the binary systems, fact which leads us to the conclusion that the inclusion phenomenon of 4-MBC in the cavity of CDs occurred.

The DSC thermograms registered in the temperature range between 30°C and 210°C showed that the complexation was complete in case of 4-MBC -β-CD and 4-MBC -HP-β-CD complexes, noticed by total disappearance of 4-MBC peak corresponding to its melting point, but was not total for the complex formed by 4-MBC with HP-α-CD.

The FT-IR spectra revealed the inclusion of photo-protector agent in the cavity of CD, and showed the formation of new hydrogen intermolecular bonds between the two molecules.

By all studied tests, it is obviously that 4-MBC forms stable complexes with β-CD, HP-β-CD, and HP-α-CD in 1:2 molar ratio, and in case of β-CDs the complexation was complete, but in case of α-CD this was partially.

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