

PREPARATION AND CHARACTERIZATION OF INCLUSION COMPLEXES FORMED BY AVOBENZONE WITH β -CYCLODEXTRIN, HYDROXYPROPYL- β -CYCLODEXTRIN AND HYDROXYPROPYL- α -CYCLODEXTRIN

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Abstract

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

Rezumat

Scopul prezentului studiu a fost confirmarea obținerii complexelor de incluziune între avobenzonă și beta-ciclodextrină, hidroxipropil-beta-ciclodextrină și hidroxipropil-alfa-ciclodextrină, prin metoda liofilizării. Complexii de incluziune au fost caracterizați din punct de vedere fizico-chimic în comparație cu simplele amestecuri fizice preparate în același raport molar de 1:2 (avobenzonă : ciclodextrină). Complexii au fost evaluați prin microscopie electronică cu baleiaj (SEM), calorimetrie diferențială dinamică (DSC) și prin spectroscopie IR (FT-IR). Complexii de incluziune ai avobenzonăi cu beta-ciclodextrine au prezentat, în toate studiile, parametri mai buni decât cei preparați cu alfa-ciclodextrină, probabil datorită moleculei avobenzonăi care are dimensiuni mai potrivite încorporării în cavitatea beta-ciclodextrinelor, decât în cea a alfa-ciclodextrinei.

Keywords: avobenzone (AVB), beta-cyclodextrin, hydroxypropyl-beta-cyclodextrin, hydroxypropyl-alfa-cyclodextrin, inclusion complex, lyophilisation

Introduction

Avobenzone (AVB) is a commonly used UVA absorber (320 – 400 nm) with peak absorption at 360 nm, one of the few active sunscreen ingredients that protect skin from the entire UVA spectrum. Avobenzone can be easily photo inactivated and studies have shown a total decomposition of avobenzone when irradiated in a cyclohexane solution for 100 h using a 450 W mercury vapour lamp. 4-tert-butylbenzoic acid and 4-methoxybenzoic acid are the major degradation products, none of which has comparable UV absorption to the parent compound. Various methods have been developed to reduce photodecomposition of avobenzone: encapsulation in liposomes, loading in solid nanoparticles, or adding a photo stabilizer into the

formulation. Complexation of avobenzone with cyclodextrins offers the possibility to enhance its stability, and decrease its degradation when exposed to light [3].

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. Their internal hydrophobic cavity allows the inclusion of lipophilic entities, resulting in enhanced water solubility, stability and bioavailability of various drugs [1, 4-6].

In the present study, we prepared by the lyophilisation method, investigated and confirmed the formation of three inclusion complexes between AVB and 2-β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-α-cyclodextrin (HP-α-CD). In order to characterize 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

AVB 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 AVB with β-CD, HP-β-CD, HP-α-CD we used the lyophilisation 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 the physical mixture between AVB and β-CD, HP-β-CD, HP-α-CD. The substances weighed according to the molar ratio of 1:2 were physically mixed for 15 minutes, at 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 AVB were added stepwise to each aqueous solution of CD. The suspension was stirred using a 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 lyophilizer [2].

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 resolution 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 resolution of 1024 pixels x 1024 pixels. Although, not all samples have enough conductivity, instead metallization, we chose the working version with low vacuum in the samples room. So, at a pressure 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 analysed 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 were calibrated with the standard material indium (99.98%, melting point: 156.65°C). The samples were analysed 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).

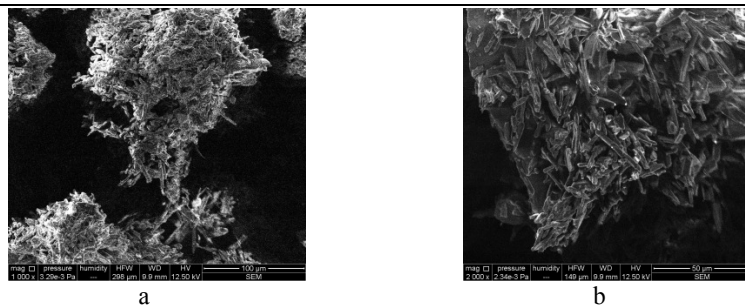
Results and Discussion

Organoleptic evaluation of the complexes

The physical mixtures obtained by mixing AVB with CDs were white, crystalline powders, odourless, with a bitter taste. The obtained complex, prepared by lyophilisation, was a very fine and smooth white powder, odourless and bitter tasted.

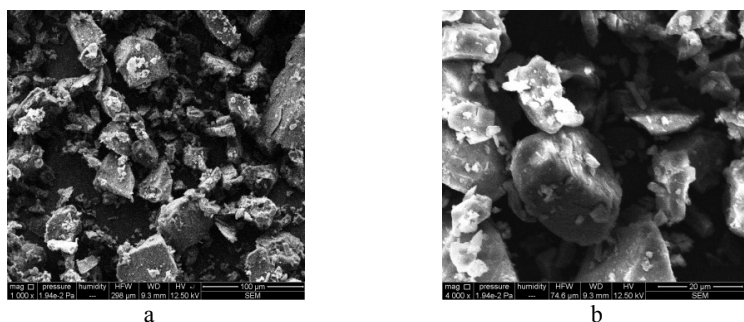
Scanning electron microscopy (SEM)

The SEM images of AVB, β-CD, HP-β-CD, HP-α-CD, their physical mixtures, and their lyophilisation complex are shown in figures 1 to 10. 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 1.**

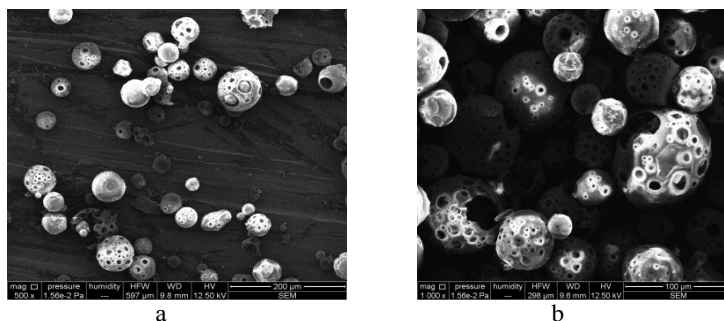
The SEM images for AVB, magnification 1000x (a), 2000x (b)

AVB is characterized by the presence of homogenous crystalline particles, with various sizes between 5 and 30 μm .

**Figure 2.**

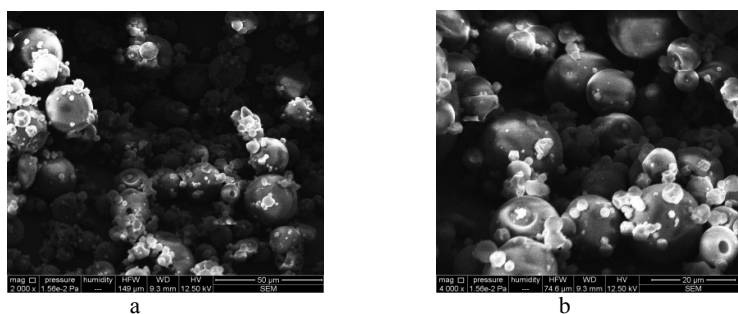
The SEM images for β -CD, magnification 1000x (a), 4000x (b)

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

**Figure 3.**

The SEM images for HP- β -CD, magnification 500x (a), 1000x (b)

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

**Figure 4.**

The SEM images for HP- α -CD, magnification 2000x (a), 4000x (b)

HP- α -CD is an amorphous powder, composed of

round particles, with a diameter between 2 and 20 μm .

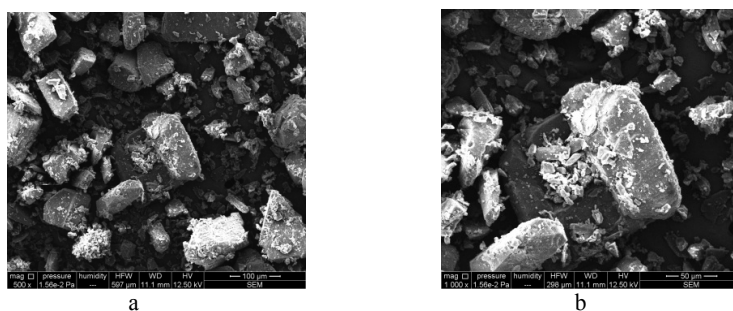


Figure 5.

The SEM images for AVB- β -CD physical mixture, magnification 500x (a), 1000x (b)

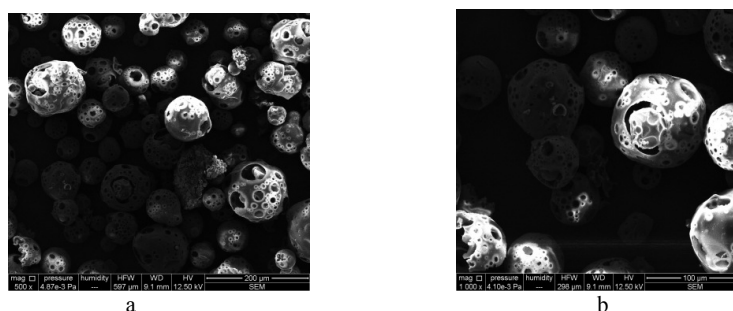


Figure 6.

The SEM images for AVB-HP- β -CD physical mixture, magnification 500x (a), 1000x (b)

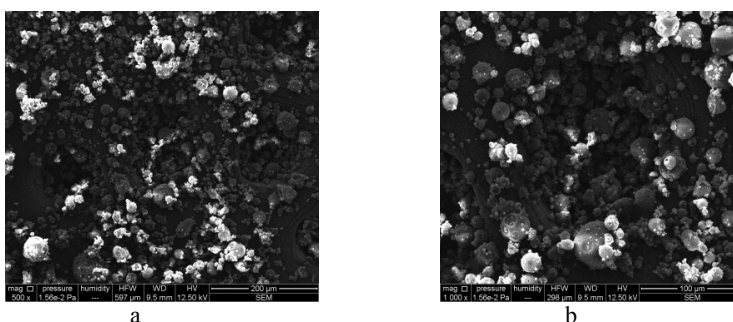


Figure 7.

The SEM images for AVB-HP- α -CD physical mixture, magnification 500x (a), 1000x (b)

The SEM images for the AVB- CDs physical mixtures shown the characteristics of AVB 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.

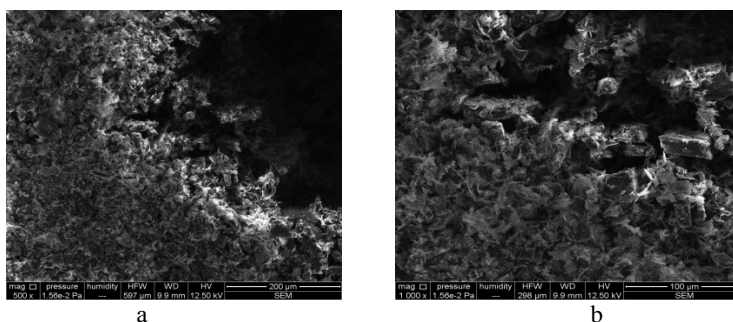
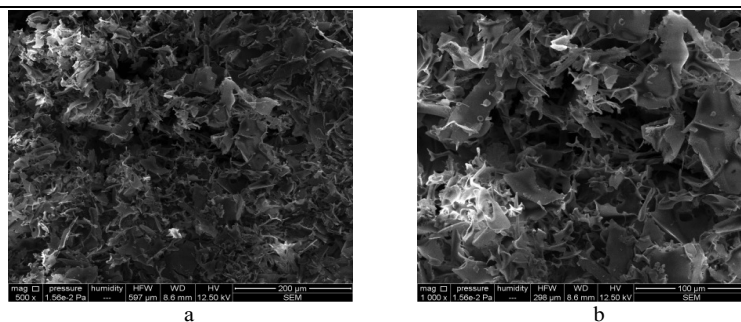
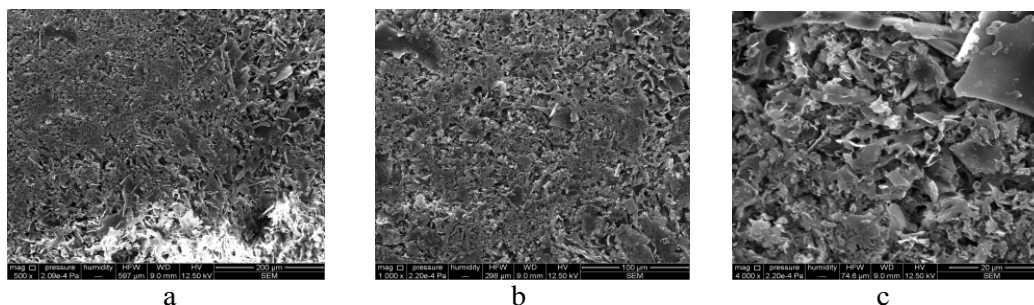


Figure 8.

The SEM images for AVB- β -CD lyophilisation inclusion complex, magnification 500x (a), 1000x (b)

**Figure 9.**

The SEM images for AVB-HP-β-CD lyophilisation inclusion complex, magnification 500x (a), 1000x (b)

**Figure 10.**

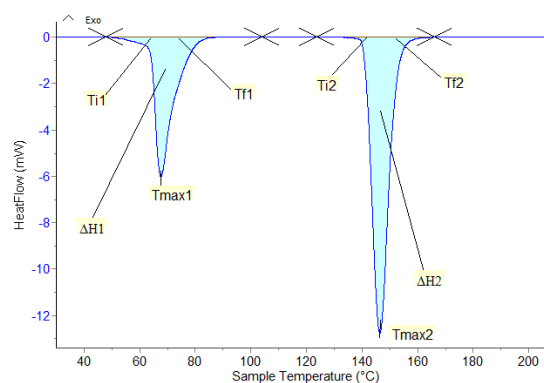
The SEM images for AVB-HP-α-CD lyophilisation inclusion complex, magnification 500x (a), 1000x (b), 4000x (c)

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

The SEM images of lyophilisation inclusion complexes clearly showed the characteristic morphology as small sized particles forming homogenous aggregates, indicating the existence of an amorphous product with presence of single component in the complex, this suggesting optimum complexation.

Differential scanning calorimetry (DSC)

The DSC thermogram of AVB (Figure 11) showed an endothermic peak at 85.45°C, corresponding to its melting point. The shape of the plot revealed the characteristics of a crystalline substance.

**Figure 11.**

The obtaining of DSC parameters

Figure 11 shows the way that DSC parameters are obtained, and Table 1 shows the DSC thermogram results for the studied samples.

Table I
The DSC thermogram results

Sample	T _{il} (°C)	T _{max1} (°C)	T _{fi} (°C)	ΔH ₁ (J/g)	T _{i2} (°C)	T _{max2} (°C)	T _{ff} (°C)	ΔH ₂ (J/g)
AVB	82.0	85.4	87.9	70.0	-	-	-	-
B-CD	111.9	133.9	146.0	222.5	-	-	-	-
HP-β-CD	59.1	109.2	163.2	166.1	-	-	-	-
HP-α-CD	57.6	109.8	162.6	175.0	-	-	-	-
AVB-β-CD physical mixture	81.2	83.7	86.8	7.3	116.5	135.1	150.6	149.5
AVB-HP-β-CD physical mixture	70.4	84.4	-	-	-	98.4	107.6	149.2*
AVB-α-CD physical mixture	80.7	84.0	87.2	16.11	93.7	123.3	149.2	42.5
AVB-β-CD complex	79.7	84.1	86.3	11.0	119.8	134.8	147.1	70.6
AVB-HP-β-CD complex	78.6	84.3	-	-	-	103.3	150.0	155.5*
AVB-HP-α-CD complex	76.8	81.6	85.7	4.8	87.6	98.9	112.2	9.7

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, 133.95°C for HP- β -CD, and 109.28°C for HP- α -CD. The shape of the plot is characteristic for the crystalline powder in the case of β -CD, and for the amorphous substances in the case of HP- β -CD and HP- α -CD.

The DSC thermograms of AVB-CDs physical mixtures showed, each of them, two endothermic peaks, one characteristic for AVB around 85°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 substances interacted, but the complexation phenomenon didn't occur.

The DSC thermograms of AVB-CDs lyophilisation inclusion complexes showed only one endothermic prominent peak: at 116.58°C with an endothermic effect of 1.79 J/g for the AVB- β -CD complex, at 98.42°C with an endothermic effect of 64.96 J/g for the AVB-HP- β -CD complex, and for the AVB-HP- α -CD complex two peaks were registered at 98.56°C with an endothermic effect of 9.79 J/g, and the other one at 81.61°C with an endothermic effect of 4.86 J/g. This last peak is characteristic for AVB, but has a much lower intensity. For the AVB- β -CD and AVB-HP- β -CD complexes it is remarkable the complete disappearance of the endothermic peak corresponding to AVB, and the DSC curves have the characteristic shape of the amorphous substances, by all these proving the complete

formation of the inclusion complex. In the meantime it is noticed that in the case of AVB-HP- α -CD complex, the complexation of AVB occurred, but was not total.

Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra of AVB presented two bands characteristic for the two carbonyl groups, at 1599 cm^{-1} and 1583 cm^{-1} , and the band produced by the methoxy group at 1256 cm^{-1} . The spectra of β -CD showed a large band at 3326 cm^{-1} produced by the O-H stretching vibration, and an intense band at 1021 cm^{-1} produced by the C-O bond's vibration.

The FT-IR spectra of HP- β -CD and HP- α -CD are very similar to that of β -CD presenting a large band at 3336 cm^{-1} , and 3335 cm^{-1} respectively, due to the O-H stretching vibrations, and another strong band at 1022 cm^{-1} , and 1023 cm^{-1} respectively, 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.

The FT-IR spectrum of AVB- β -CD inclusion complex (Figure 12) 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 3326 cm^{-1} in β -CD to a value of 3315 cm^{-1} , probably due to the new formed hydrogen intermolecular bonds. Instead, the characteristic spectral portion for AVB significantly decreases in intensity, mainly in the 1600-1200 cm^{-1} zone, due to its inclusion in the CD molecule.

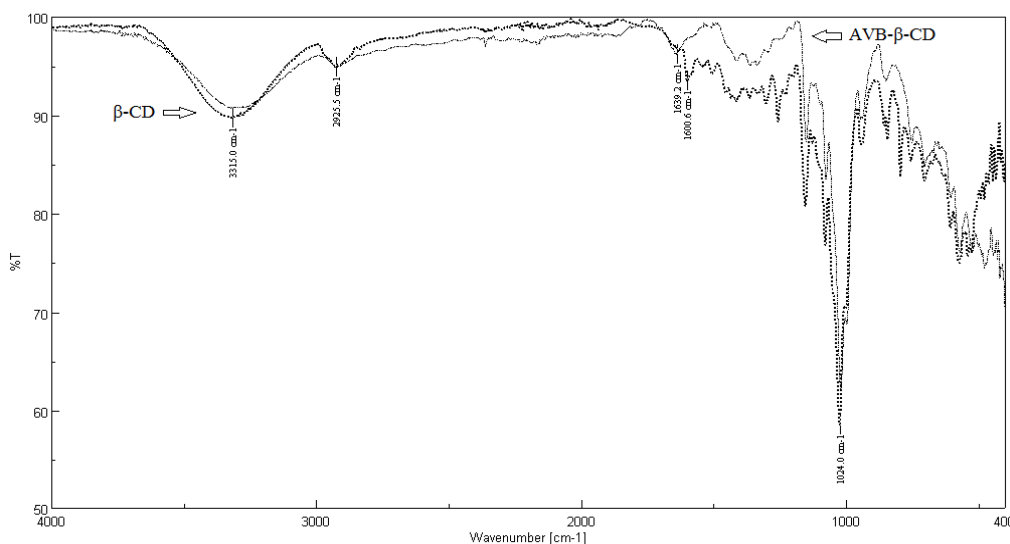
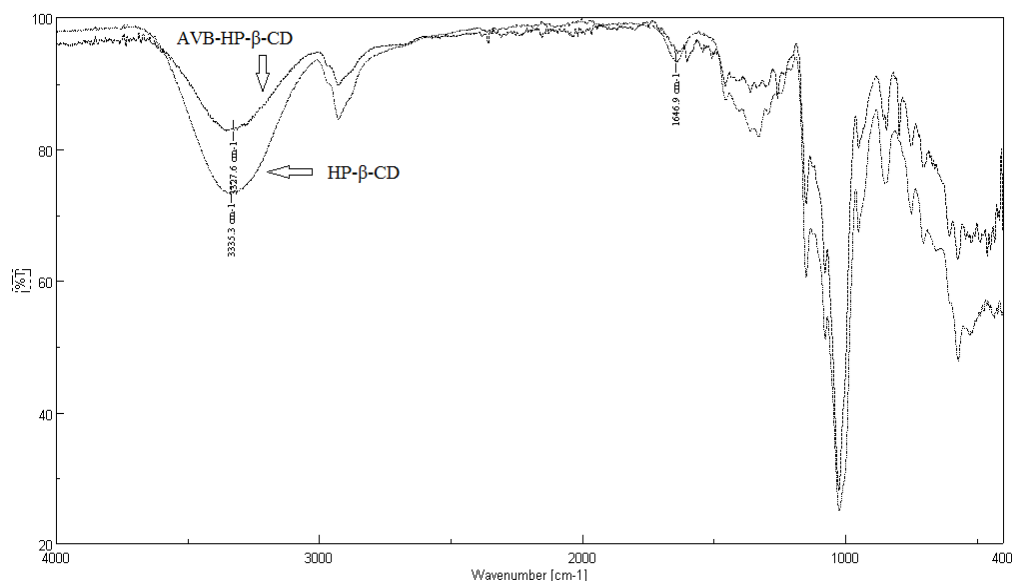


Figure 12.

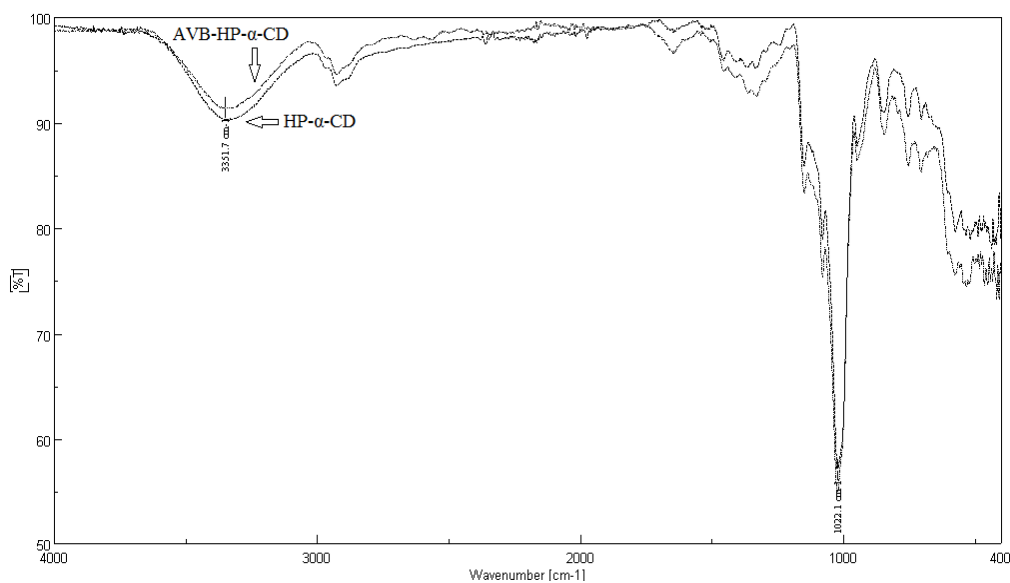
The FT-IR spectra of AVB- β -CD inclusion complex and of β -CD

The FT-IR spectra of AVB-HP- β -CD inclusion complex (Figure 13) presents a high resemblance with the one of AVB-HP- α -CD inclusion complex (Figure 14), the O-H stretching vibration

decreasing from 3335 cm^{-1} in CD to a value of 3328 cm^{-1} , similar to the AVB- β -CD complex and the characteristic spectral portion for AVB significantly decreases in intensity.

**Figure 13.**

The FT-IR spectra of HP- β -CD and of AVB-HP- β -CD inclusion complex

**Figure 14.**

The FT-IR spectra of HP- α -CD and AVB-HP- α -CD lyophilisation inclusion complex

Conclusions

The studied inclusion complexes between AVB and β -CD, HP- β -CD, and HP- α -CD were prepared in a molar ratio of 1:2, by the lyophilisation method, when amorphous powders were obtained. In order to compare the results for the complexes, we prepared three physical mixtures between AVB 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 mixtures, 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 AVB 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 the case of AVB- β -CD and AVB-HP- β -CD complexes, noticed by the total disappearance of AVB peak corresponding to its melting point, but was not total for the complex formed by AVB with HP- α -CD.

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

By all performed tests, it is obvious that AVB forms stable complexes with β -CD, HP- β -CD, and

HP- α -CD in 1:2 molar ratio, and in the case of β -CDs the complexation was complete, but in the case of α -CD this phenomena was partial.

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