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INFLUENCE OF THE METHOD OF PREPARATION ON PHYSICOCHEMICAL CHARACTERISTICS OF 1:1 SIMVASTATIN-β-CYCLODEXTRIN INCLUSION COMPLEX

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ABSTRACT
The aim of the present study was to confirm the formation of 1:1 molar ratio inclusion complexes between the practically insoluble drug, simvastatin (SV) and beta-cyclodextrin (β-CD) in aqueous solution and in solid states by different techniques. Complexation in solution was evaluated using Higuchi-Connors phase solubility studies. Phase solubility diagram was classified as A_L type, indicating the formation of 1:1 molar ratio inclusion complex, with an apparent stability constant (K_{st}) of 460 M^{-1}. The inclusion complexes in solid state were prepared by co-precipitation and lyophilization techniques, and were subjected to physicochemical characterizations in comparison with the simple physical mixture prepared in the same 1:1 molar ratio. The complexes were evaluated by the scanning electron microscopy (SEM), powder X-ray diffraction, differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FT-IR). All these studies showed the formation of 1:1 molar ratio inclusion complex between simvastatin and beta-cyclodextrin by both preparation methods used, but by liophilization the complexation proved to be complete.
Keywords: simvastatin, beta-cyclodextrin, inclusion complex, lyophilization, phase solubility diagrams.

INTRODUCTION
Simvastatin (SV) is a cholesterol lowering agent, derived structurally from a fermentation product of Aspergillus terreus, widely used to treat hypercholesterolemia. It is potent inhibitor of 3-hydroxyl-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, this conversion being an early and rate-limiting step in the biosynthesis of cholesterol. (Seoung, 2007) SV, an inactive lactone, is converted to corresponding β, δ-dihydroxy acid in liver by cytochrome P450 (CYP) 3A after oral administration. (Shiralashetti, 2010). SV is a white, nonhygroscopic, crystalline powder, being practically insoluble in water and poorly absorbed from the gastrointestinal tract. Also, SV is a very unstable substance, being easily degraded in an oxydation process accelerated by humidity, high temperatures, light and oxydising agents. Therefore, it is cery important to introduce effective methods to enhance the solubility and dissolution rate of drug. Complexation of SV with β-cyclodextrins offers this possibility, and also increases the stability of SV (McClelland, 1991).

Cyclodextrins (CDs) are cyclic oligosaccharides produced by enzymatic degradation of the starch, by the action of cyclodextrin-glycosyl-transferase derived from Bacillus macerans. 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 (Loftsson, 2007).

In the present study, we prepared by co-precipitation and lyophilization methods, investigated and confirmed the formation of an inclusion complex between SV and β-cyclodextrin (β-CD), comparing the obtained results.

MATERIALS AND METHODS
Materials
SV was obtained from Biocon Limited Biopharmaceuticals, India, and β-CD was purchased from Sigma -Aldrich Chemie GmbH, Germany.

The methanol, ethanol 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
Phase solubility diagram

Initially, an aqueous solution (0.0145 M) of β-CD was prepared, devided in five vials in increased volumes from 0.5 ml to 5 ml, and completed with water to 5 ml. Then, excess amounts of SV (10 mg) were added to this solutions containing various concentrations of β-CD. The vials were shaken for 24 h, at 750 rpm, at room temperature 25 ± 2 °C, using the Heidolph Vibramax

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100 shaker. When the equilibrium was reached, after six hours of repose at the same temperature, the samples were withdrawn and filtered through a 0.45 μm nylon filter membrane (Whatman® Puradisc^{TM}), suitably diluted with water, then the absorbance at 240 nm against a water blank was measured. The concentration of dissolved SV was determined spectrophotometrically using the Perkin - Elmer LAMBDA 2 UV-VIS spectrometer.

The experiments were conducted in duplicate.

From the Higuchi and Connors phase solubility diagrams (Higuchi, 1965) we calculated the apparent solubility constant (K_{st}), according to the hypothesis of a complex formation in 1:1 stoichiometric ratio, using the following equation:

$$K_{st} = \frac{\text{slope}}{S_0 (1 - \text{slope})}$$

where S_0 = the intrinsec solubility of SV in the absence of β-CD.

The slope is obtained from the initial straight line portion of the plot of SV against β-CD concentration. [8]

Preparation of the complexes

In order to obtain the inclusion complexes of SV with β-CD, we used two methods of complexation in solution: co-precipitation and liophilization, and to compare the results of characterisation tests of the complexes we prepared simple physical mixture in the same ratio.

Preparation of physical mixture between SV and β-CD

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

Preparation of the complexes by co-precipitation method

0.5 mmol of β-CD were dissolved in 100 ml of distilled water. 15 ml of 96 % ethanolic solution containing 0.5 mmol of SV was added stepwise stepwise to the aqueous solution of β-CD. The suspension was stirred with Heidolph MR 3001K magnetic stirrer, for 6 h, at 750 rpm, at room temperature, then filtered, and the powder was dried at 25°C in an exsiccator.

Preparation of the complexes by lyophilization method

The same preparation steps were followed as for the co-precipitation method, and after stirring 6 h, the final suspension was lyophilized at -60°C, for 12 h, using the Christ ALPHA 1 – 2, B Braun Biotech International, Germany liophilizer.

Both of the inclusion complexes obtained, and also the physical mixture were were evaluated by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffraction, 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 10-15 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 500x-10000x, 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.

Powder X-ray diffraction (PXRD)

Powder X-ray diffraction patterns of samples were obtained with the Panalytical X'Pert Pro MPD (Netherlands) powder X-ray diffractometer, with Bragg – Brentano geometry in 2θ, using a Ni filtered Cu-K_{α} beam X-ray as the source of radiation. It was operated at the voltage of 45 kV and a current of 40 mA. All samples were measured in the angle range between 5° and 50°. Each sample was placed in the cavity of an aluminum sample holder flattened with a glass slide to present a good surface texture and inserted into the sample holder. In order to measure the powder pattern, the sample holder and detector were moved in a circular path to determine the angles of scattered radiation and to reduce preferred sample orientation. The compounds were analyzed in duplicate.

Differential scanning calorimetry (DSC)

DSC analyses of the samples were performed on DSC Q 2000 V 24,9 Build 121, produced by TA Instruments, USA, 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 a helium atmosphere with a flow rate of 50.0 ml/min, in a temperature range of 20°C -150 °C. As reference an empty 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 with diamond crystal, on a spectral range of 4000-400 cm^{-1} and a resolution of 4 cm^{-1}. The compounds were analyzed in duplicate. The spectra are presented in transmittance percentages (T%) over wavelength (cm^{-1}).

RESULTS AND DISCUSSION

Phase solubility diagram

According to the classification introduced by Higuchi and Connors, the phase solubility diagram registered for the simvastatin - β-CD inclusion complex (fig. 1) was linear A_L type which indicates the forming of a water soluble complex. A_L type diagrams are obtained when the apparent solubility of the active increases linear with increasing CD concentration over the entire concentration range. Calculated regression parameters, and also the shape of the plot allowed us to assume that simvastatin - β-CD inclusion complex, formed in 1:1 molar ratio, the slope being < 1, is present in the aqueous solution.

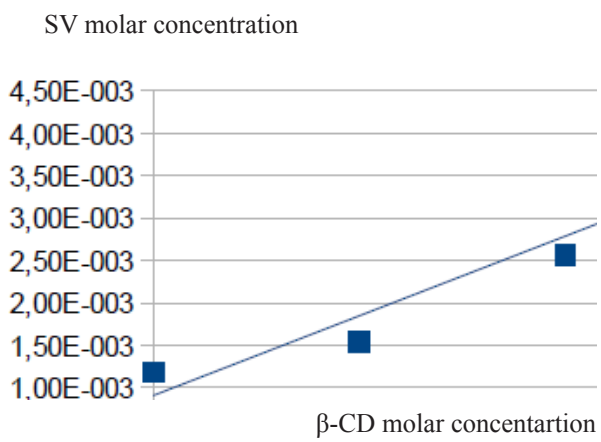
Table 1 shows the experimental data obtained, the regression parameters, and the apparent complexation constant (K_{st}) for the simvastatin - β-CD inclusion complex.

Table 1 – The phase solubility diagram parameters of the inclusion complex formed between SV and β-CD

β-CD molar concentration	Absorbance	SV molar concentration	Slope	Intrinsec solubility	Stability constant
0,0014	0,167	1,19E-003	3,84E-001	5,14E-004	460 M^{-1}
0,0029	0,217	1,54E-003			
0,0058	0,361	2,57E-003			
0,0087	0,559	3,97E-003			
0,0145	1,257	8,94E-003			

The phase solubility diagram of SV with β-CD is shown in figure 1.

Figure 1 – The phase solubility diagram of SV - β-CD inclusion complex



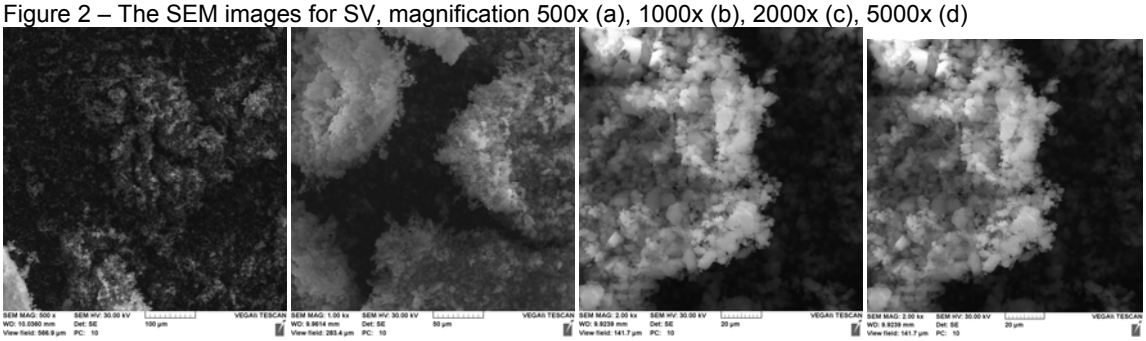
Organoleptic evaluation of the complexes

The physical mixture obtained by mixing SV with β-CD was a white, crystalline powder, odorless, with a bitter taste. The complex prepared by co-precipitation method was white, odorless, bitter tasted, amorphous powder. By lyophilization we obtained a very fine and smooth white powder, odorless and bitter tasted.

Scanning electron microscopy (SEM)

The SEM images of SV, β-CD, SV - β-CD physical mixture, SV - β-CD co-precipitation complex, and SV - β-CD liophilization complex are shown in figures 2, 3, 4, 5 and 6. The images were captured with a magnification between 500x and 5000x, so the uniformity on a large quantity of powder and also the details of the samples, can be seen.

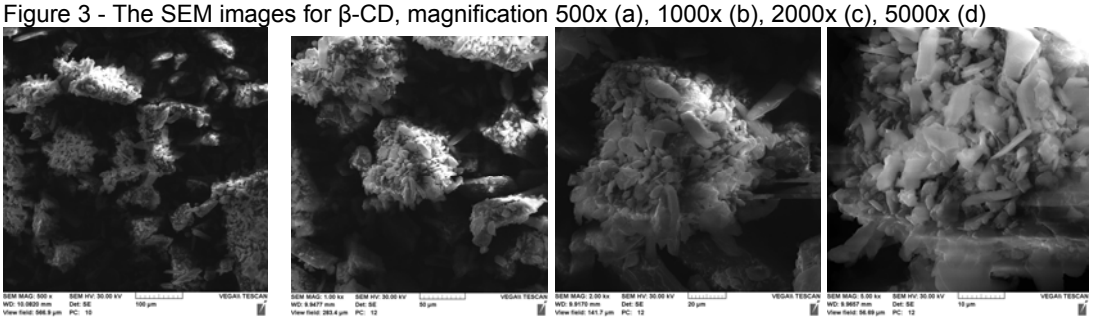
Figure 2 – The SEM images for SV, magnification 500x (a), 1000x (b), 2000x (c), 5000x (d)



a b c d

SV is characterized by the presence of homogenous crystalline particles, with various sizes between 3 and 10 μm.

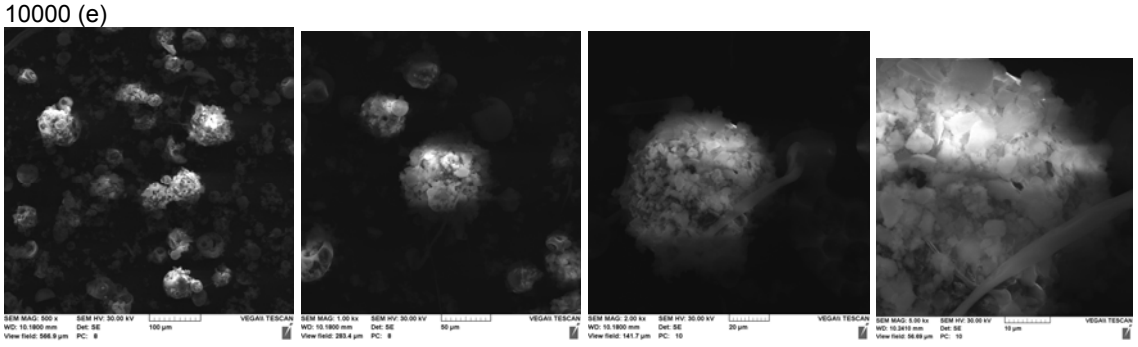
Figure 3 - The SEM images for β-CD, magnification 500x (a), 1000x (b), 2000x (c), 5000x (d)



(a) (b) (c) (d)

β-CD has, also, different shapes of crystalline particles, with homogenous surface, with sizes between 5 and 10 an 10 μm.

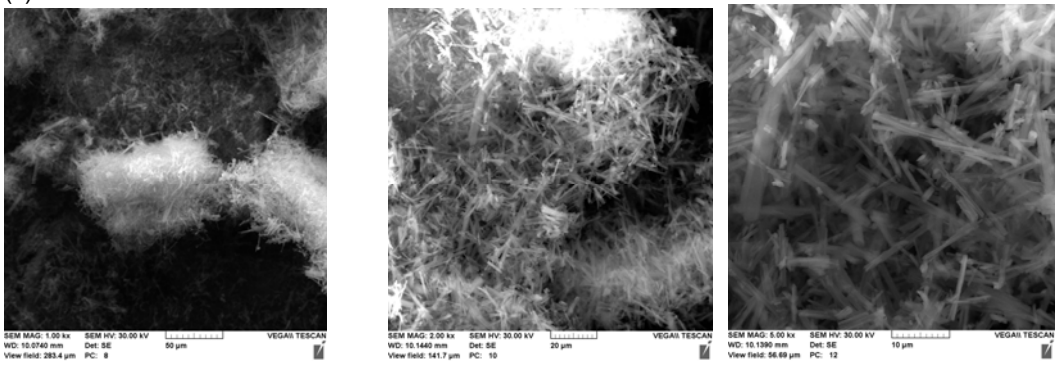
Figure 4 - The SEM images for SV- β-CD physical mixture, magnification 500x (a), 1000x (b), 2000x (c), 5000x (d), 10000 (e)



(a) (b) (c) (d)

The SEM images for the SV- β-CD physical mixture clearly shown the crystalline structures of SV and β-CD, but the crystals of drug and the particles of CD were seen adhering to their surface, this confirming the affinity between substrates.

Figure 5 - The SEM images for SV-β-CD co-precipitation inclusion complex, magnification 1000x (a), 2000x (b), 5000x (c)



(a) (b) (c)

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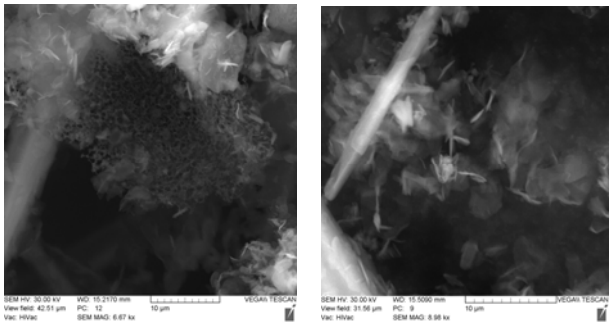
The SEM pictures for the SV-β-CD co-precipitation inclusion complex shown the formation of a new slightly amorphous structure, the complex appearing under the form of some very fine and irregular crystals, with a obviously decrease of particles size, and a partial loss of crystallinity towards the initial components, but it can be still seen SV or β-CD isolated particles. This indicates the fact that by this co-precipitation method the complexation is taking place, but is not total.

Figure 6 - The SEM images for SV-β-CD lyophilization inclusion complex, magnification 6670 x (a), 8980x (b)



(a) (b)

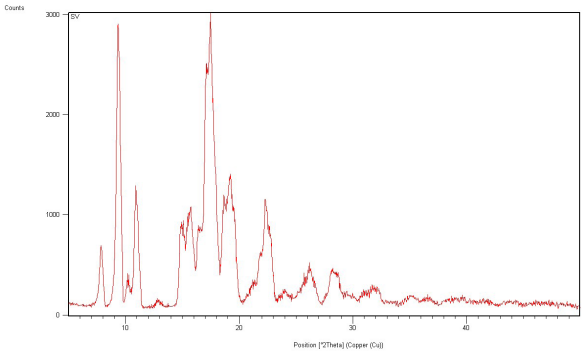
In contrast with the co-precipitation inclusion complex, the lyophilization complex shown a drastic change in the morphology and shape of the particles. In the lyophilization case, it was no longer possible to distingish the two components, SV and β-CD, revealing a strong interaction between the drug and CD in these systems.

The SEM images of lyophilization inclusion complex 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.

Powder X-ray diffraction (PXRD)

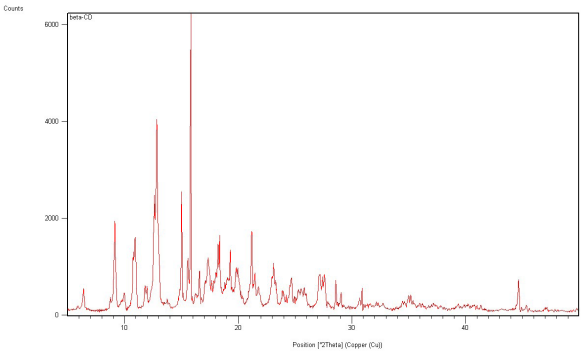
Powder X-ray diffraction patterns of SV, β-CD, SV-β-CD physical mixture, SV-β-CD co-precipitation complex, and SV-β-CD liophilization complex are presented in figures 7, 8, 9, 10 and 11.

Figure 7 – The powder X-ray diffraction pattern of SV



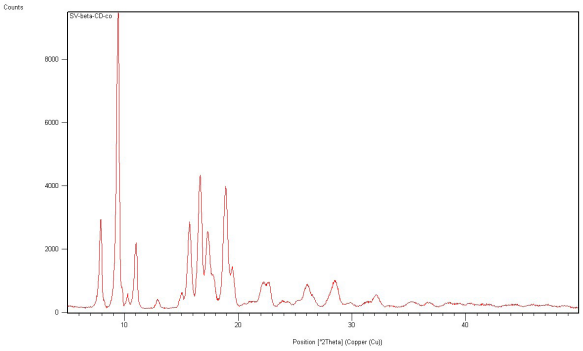
The powder X-ray diffraction pattern of SV shows that the drug is present as a crystalline form, by the presence of several distinct peaks at a 2θ diffraction angle of 7,87°, 9,31°, 10,98°, 12,86°, 15,61°, 16,49°, 17,28°, 18,85°, 22,56°, 25,93°, 28,37° şi 31,94°.

Figure 8 – The powder X-ray diffraction pattern of β-CD



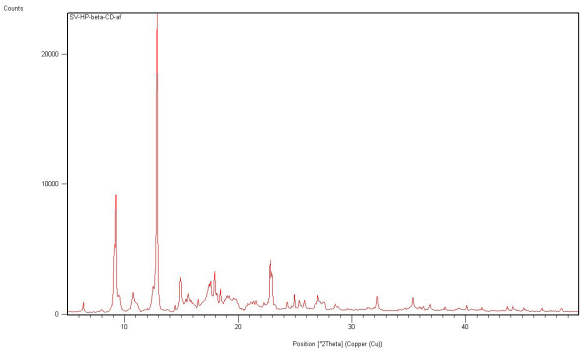
The presence of a series of intense peaks in the powder X-ray diffraction pattern of β-CD also indicates the fact that the substance has a crystalline nature.

Figure 9 – The powder X-ray diffraction pattern of SV-β-CD physical mixture



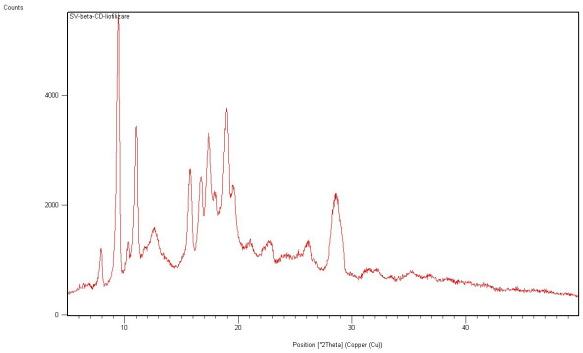
The powder X-ray diffraction pattern of SV-β-CD physical mixture presents the overlap of the spectra registered for both components, not showing the formation of a new compound with a new structure. Yet, the powder X-ray diffraction pattern of the physical mixture is more like the one corresponding to SV.

Figure 10 – The powder X-ray diffraction pattern of SV-β-CD co-precipitation inclusion complex



The powder X-ray diffraction pattern of SV-β-CD co-precipitation inclusion complex shown the presence of a new compound with a lot of β-CD characteristics, but still mentaining some of SV peaks.

Figure 11 – The powder X-ray diffraction pattern of SV-β-CD lyophilization inclusion complex

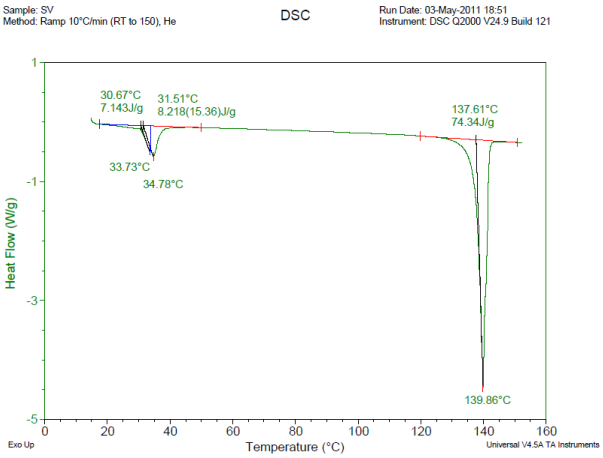


The SV - HP-β-CD lyophilization inclusion complex presented the powder X-ray diffraction pattern with less peaks low intensity, keeping somehow the base characteristics of the initial substances, but a new system with an amorphous structure was formed.

Differential scanning calorimetry (DSC)

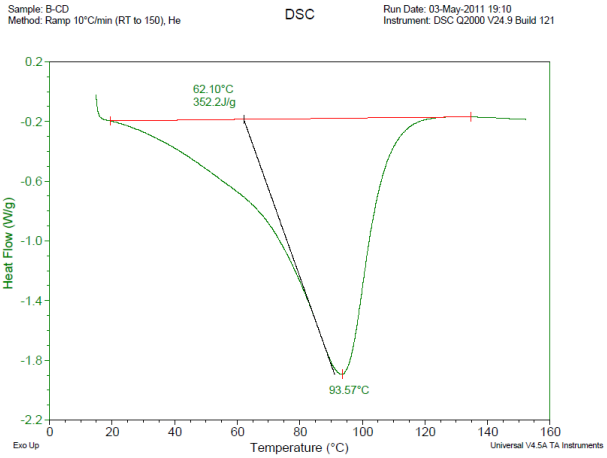
The DSC thermogram of SV (figure 12) shown an endothermic peak at 139,86°C, corresponding to its melting point, and a second endothermic peak at 34,78°C due to the presence of the water absorbed by the sample. The shape of the plot reveals the characteristics of a crystalline substance.

Figure 12 – The DSC thermogram of SV



The DSC thermogram of β-CD showed an endothermic peak at 93,57°C (figure 13). The endothermic peaks with a low intensity and appeared at low temperatures, under 100°C, are due to the water absorbed by the sample. [5]

Figure 13 – The DSC thermogram of β-CD



The DSC thermogram of SV-β-CD physical mixture (figure 14) shown two endothermic peaks, one characteristic for SV at 140.13°C, but with a low intensity, with an endothermic effect of 19.86 J/g, and the other one at 67.84°C, due to the water loss, with an endothermic effect of 135.80 J/g. This result proves that the two substances interacted, but the complexation phenomenon didn't occur.

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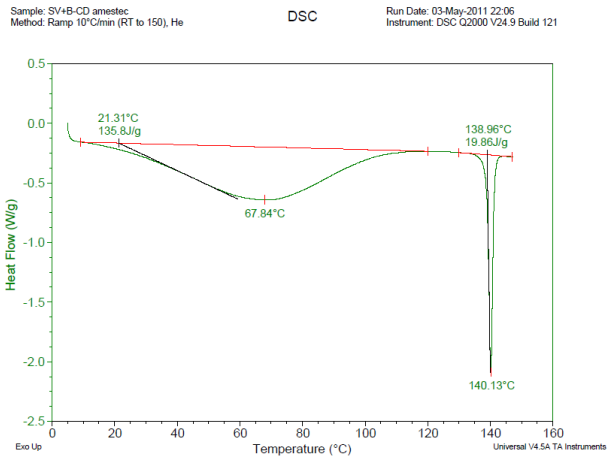
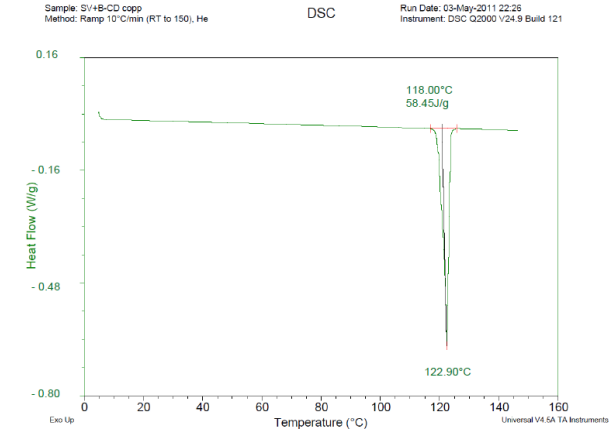
Figure 14 – The DSC thermogram of SV-β-CD physical mixture



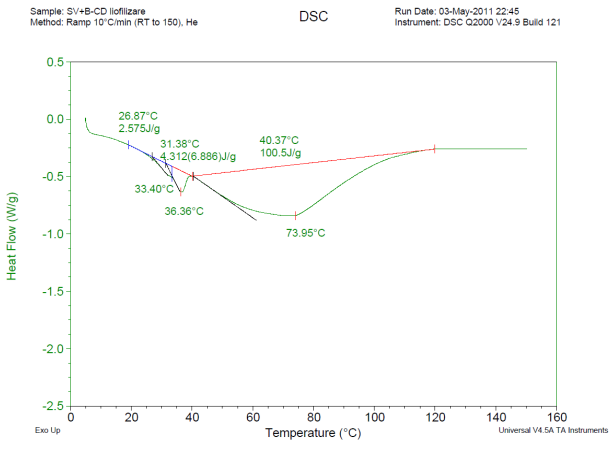
For the SV-β-CD co-precipitation inclusion complex, the DSC thermogram (figure 15) is characterized by the presence of only one endothermic peak at 122.90°C with an endothermic effect of 58.45 J/g similar with the one of SV, but of a much lower intensity and at a smaller value of temperature.

Figure 15 – The DSC thermogram of SV-β-CD co-precipitation inclusion complex



The DSC thermograms of SV-β-CD lyophilization inclusion complex (figure 16) showed only one endothermic peak at 73.95°C with a large endothermic effect of 100.50 J/g, due to the release of water molecules from the structure, remarkable being the complete disappearance of endothermic peak corresponding to SV. The DSC curve has the characteristic shape of the amorphous substances, by all these proving the forming of the inclusion complex.

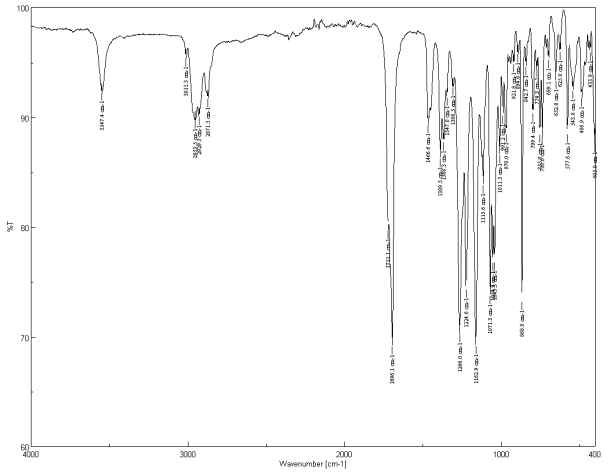
Figure 16 – The DSC thermogram of SV-β-CD lyophilization inclusion complex



Fourier-transform infrared spectroscopy (FT-IR)

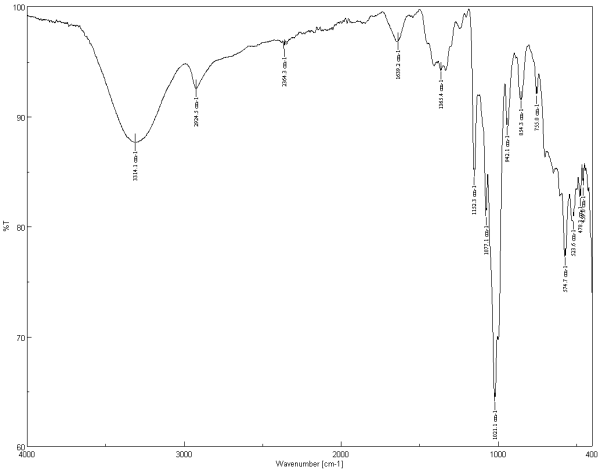
The FT-IR spectra of SV (figure 17) showed the presence of the following major peaks: 3547 cm^{-1} (O–H stretching vibration), 3011 cm^{-1}, 2954 cm^{-1}, and 2872 cm^{-1} (C–H stretching vibrations), 1722 cm^{-1} (stretching vibration of lactone carbonyl) and 1696 cm^{-1} (stretching vibration of ester carbonyl group), 1266 cm^{-1} (lactone –C–O–C vibration).

Figure 17 – The FT-IR spectra of SV



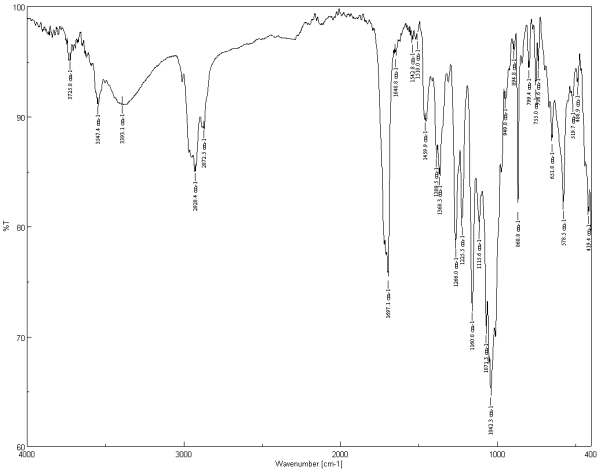
The FT-IR spectra of β-CD (figure 18) showed a large absorbtion band at 3314 cm^{-1} (O–H stretching vibrations), 2924 cm^{-1} (C–H stretching vibrations) and at 1021 cm^{-1} a very strong band produced by the C–O bond's vibration.

Figure 18 – The FT-IR spectra of β-CD



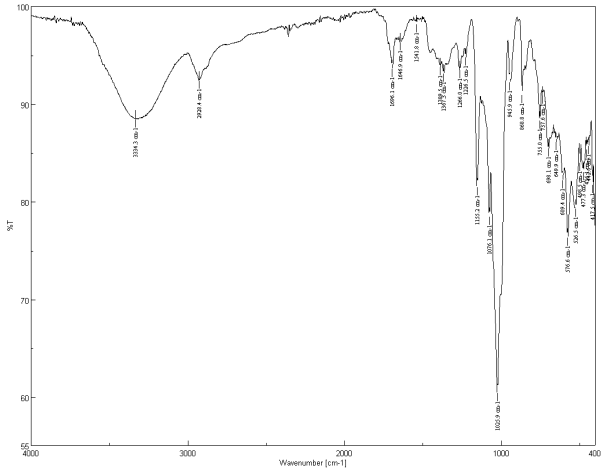
The FT-IR spectra of SV-β-CD physical mixture (figure 19) is the arithmetic sum of IR spectra of the components, without changes in the wavelengths of spectra's maximum.

Figure 19 – The FT-IR spectra of SV-β-CD physical mixture



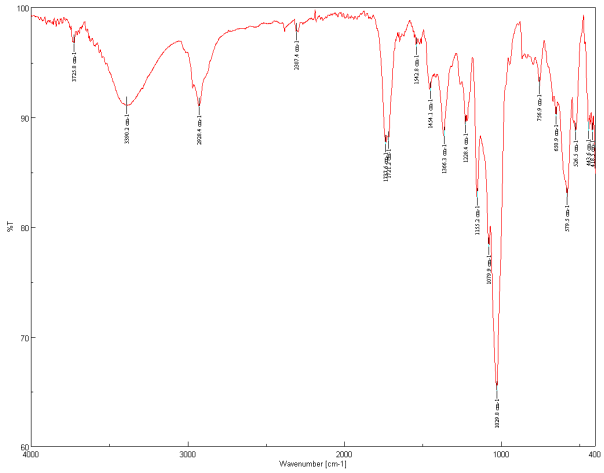
The FT-IR spectra of SV-β-CD co-precipitation inclusion complex is presented in the figure 20. The characteristic bands of the two carbonyl groups of SV have smaller transmissions (T%) in the complex and higher wavelength values 1722 cm^{-1} and 1704 cm^{-1}. A higher wavelength can be also observed for the C–O vibration (1026 cm^{-1}) and for the O–H stretching vibrations (3334 cm^{-1}), proving the formation of new weak bonds between β-CD and SV in the structure of the complex.

Figure 20 – The FT-IR spectra of SV-β-CD co-precipitation inclusion complex



In the FT-IR spectra of SV-β-CD liophylization inclusion complex (figure 21) significant differences compared with SV, proving the formation of the complex. The characteristic bands of the two carbonyl groups of SV have smaller transmissions (T%) in the complex and higher wavelength values, 1737 cm-1 and 1721 cm-1. A higher wavelength can be also observed for the C–O vibration (1030 cm-1) and for the O–H stretching vibrations (3390 cm-1), proving the formation of new weak bonds between β-CD and SV in the structure of the complex.

Figure 21 – The FT-IR spectra of SV-β-CD lyophilization inclusion complex



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CONCLUSIONS

The phase solubility diagram of SV in various concentrations of β-CD was linear and the type of the complex is classified as A_L. The aqueous solubility of SV was increased linearly as a function of the concentration of β-CD, with a slope of < 1 indicating that the increase in solubility was due to the formation of 1:1 molar ratio complex. The value of stability constant (K_{st}) confirms the fact that the final complex is relatively stable.

The studied inclusion complexes between SV and β-CD were prepared in a molar ratio of 1:1, by two methods, co-precipitation and lyophilization, when amorphous powders were obtain. In order to compare the results of the complexes analyses, we prepared a physical mixture between SV and β-CD, also in a molar ratio of 1:1, when a crystalline powder was obtained.

By evaluating the morphology of the two 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 SV in the cavity of β-CD occurred. We also observed that in the co-precipitation complex the characteristics of the crystalline substances remained, meantime the liophylization complex had a completely new structure with an amorphous texture.

The powder X-ray diffraction patterns showed that in the physical mixture is not registering important differences compared with SV, meantime by co-precipitation processes a new compound maintaining some of SV characteristic bands was obtained, but by lyophilization processes, amorphous new compound with more β-CD characteristics appeared.

The DSC thermograms registered in the temperature range between 20°C and 150°C showed that by co-precipitation method the complexation was not complete for the molar ratio of 1:1 - SV: β-CD due to the appearance of the SV characteristic peak at lower intensity and value of temperature, but by liophylization method the complexation was complete, noticed by total disappearance of SV peak corresponding to its melting point.

The FT-IR spectra revealed that the co-precipitation complex has however weaker bonds than those formed in the lyophilization complex, proved by smaller wavelength values of the two carbonyls and for the O–H stretching vibrations.

By all studied tests, it is obviously that SV forms stable and more soluble inclusion complexes with β-CD in 1:1 molar ratio, but by co-precipitation method the complexation was partially, meantime by lyophilization method this was complete.

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