

PREPARATION AND CHARACTERIZATION OF INCLUSION COMPLEXES FORMED BETWEEN SIMVASTATIN AND HYDROXYPROPYL – β – CYCLODEXTRIN

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Abstract

The aim of the present study was to evaluate the formation of inclusion complexes between simvastatin and hydroxypropyl-beta-cyclodextrin in aqueous solution and in solid state. Complexation in solution was assessed using Higuchi-Connors phase solubility studies. Phase solubility diagram was classified as A_L type, indicating the formation of a 1:1 molar ratio inclusion complex, with an apparent stability constant (K_{st}) of 169 M^{-1} . The inclusion complexes in solid state were prepared by co-precipitation and lyophilization techniques, and were subjected to physico-chemical characterization in comparison with the simple physical mixture prepared in the same 1:1 molar ratio. The complexes were evaluated by scanning electron microscopy (SEM), powder X-ray diffraction, differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FT-IR). The lyophilization inclusion complexes between simvastatin and hydroxypropyl-beta-cyclodextrin, in 1:1 molar ratio, showed better results in all the studied parameters in comparison with the complexes prepared by the co-precipitation method.

Rezumat

Scopul prezentului studiu a fost acela de a evalua formarea complexelor de incluziune între simvastatin și hidroxipropil-beta-ciclodextrină, atât în soluție apoasă, cât și în stare solidă. Complexarea în soluție a fost cercetată prin studiile de fază-solubilitate Higuchi-Connors. Diagrama de fază-solubilitate obținută este de tipul A_L , indicând formarea complexului de incluziune în raport molar de 1:1, cu o constantă aparentă de stabilitate (K_{st}) de 169 M^{-1} . Complexii de incluziune în stare solidă au fost preparați prin coprecipitare și liofilizare, apoi au fost supuși caracterizărilor fizico-chimice prin comparație cu un amestec fizic simplu, preparat în același raport molar de 1:1. Complexii au fost evaluați prin microscopia electronică cu baleiaj (SEM), difracție cu raze X, calorimetrie diferențială dinamică (DSC) și prin spectroscopie IR (FT-IR). Complexii de incluziune ai simvastatinului în hidroxipropil-beta-ciclodextrină obținuți prin liofilizare au prezentat, în toate studiile, parametri mai buni decât cei obținuți prin metoda coprecipitării.

Keywords: simvastatin, hydroxypropyl-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 a potent inhibitor of 3-hydroxyl-3-methylglutaryl-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. SV is a white, non-hygroscopic, crystalline powder, being practically insoluble in water and poorly absorbed from the gastrointestinal tract. Also, SV is easily degraded in an oxydation process accelerated by humidity, high temperatures, light and oxydizing agents. Complexation of SV with β -cyclodextrins offers the possibility to enhance the aqueous solubility and dissolution rate of the drug from its oral dosage forms, and also to increase the stability of SV. [4, 5, 7]

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. [2, 3, 6, 9]

In the present study, we obtained by the co-precipitation and lyophilization methods, investigated and confirmed, the formation of an inclusion complex between SV and 2-hydroxypropyl- β -cyclodextrin (HP- β -CD). HP- β -CD has a high aqueous solubility (45 %) and a good ability to stabilize the drug molecule. The type of complexation and complexation constant were established according to the phase solubility study, Higuchi and Connors method. [1] To characterize the properties of the complexes in solid state we used scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffraction, and Fourier-transform infrared spectroscopy (FT-IR) analyses.

Materials and methods

Materials

SV was obtained from Biocon Limited Biopharmaceuticals, India, and HP- β -CD was purchased from Sigma -Aldrich Chemie GmbH, Germany. The methanol, ethanol and distilled water were of analytical grade. For weighing the substances we used a Mettler Toledo AT261 balance (with 0.01 mg sensitivity).

Methods

Phase solubility diagram

Initially, an aqueous solution (0.1M) of HP- β -CD was prepared, divided in six vials in increased volumes from 0.25 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 HP- β -CD. The vials were shaken for 24 h, at 750 rpm, at room temperature $25 \pm 2^\circ\text{C}$, using the Heidolph Vibramax 100 shaker. When the equilibrium was reached, after six hours of repose at the same temperature, the samples were withdrawn and filtered through a $0.45\ \mu\text{m}$ nylon filter membrane (Whatman® Puradisc™), 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 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:

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

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

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

Preparation of the complexes

In order to obtain the inclusion complexes of SV with HP- 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 a simple physical mixture in the same ratio.

Preparation of the physical mixture between SV and HP- β -CD

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

Preparation of the complexes by the co-precipitation method

0.5 mmols of HP- β -CD were dissolved in 100 mL of distilled water. 15 mL of 96% ethanolic solution containing 0.5 mmols of SV was added

stepwise to the aqueous solution of HP- β -CD. The suspension was stirred using the 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 the 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 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 μ A, a "probe current", PC, between 1:8 - 1:12, current absorbed up to $\approx$ 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 500x-10000x, with a fixed digitization rezolution of 1024 pixels x 1024 pixels. Although, not all samples had enough conductivity, instead metallization, we choose to work in low vacuum conditions in the samples room. So, at a 10 Pa presure in the samples room, 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 was 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 ($95^\circ\text{C}/\text{min}$), 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 $10^\circ\text{C}/\text{min}$, in a helium atmosphere with a flow rate of $50.0\text{mL}/\text{min}$, in a temperature range of 20°C - 150°C . As reference an empty aluminium pan was used. Temperature and enthalpy were 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 $4000\text{--}400\text{ cm}^{-1}$ 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 - HP- 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 ingredient increases linearly with the increase of CD concentration over the entire concentration range. Calculated regression parameters (slope < 1), and also the shape of the plot allowed us to assume that an 1:1 simvastatin - HP- β -CD inclusion complex formed is present in the aqueous solution.

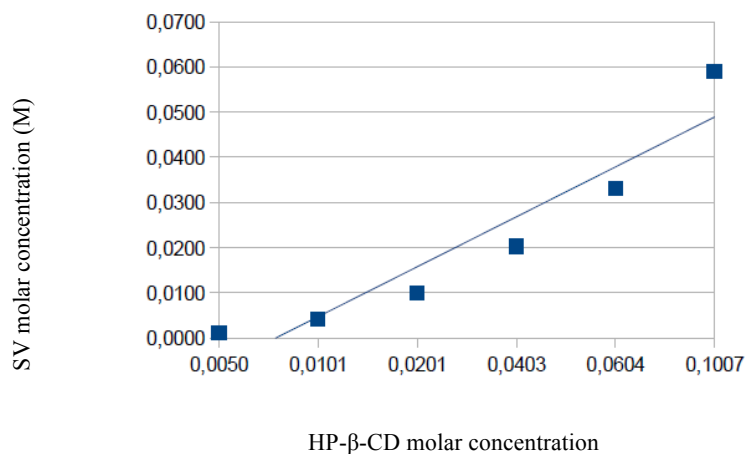
Table I shows the experimental data obtained, the regression parameters, and the apparent complexation constant (K_{st}) for the simvastatin - HP- β -CD inclusion complex.

Table I

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

HP- β -CD molar concentration (M)	Absorbance	SV molar concentration (M)	Slope	Intrinsic solubility	Stability constant
0.0038	0.717	5.10E-003	1.1664	-0.0011	169 M ⁻¹
0.0075	1.053	7.49E-003			
0.0151	2.031	1.44E-002			
0.0754	2.451	1.74E-002			

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

**Figure 1**

The phase solubility diagram of SV - HP- β -CD inclusion complex

Organoleptic evaluation of the complexes

The physical mixture obtained by mixing SV with HP- β -CD was a white, crystalline powder, odorless, with a bitter taste. The complex prepared by co-precipitation method was a 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, HP- β -CD, SV - HP- β -CD physical mixture, SV - HP- β -CD co-precipitation complex, and SV - HP- β -CD liophilization complex are shown in figures 2, 3, 4, 5 and 6. The images were captured using a magnification between 500x and 5000x, so the uniformity on a large quantity of powder and also the details of the samples, can be observed.

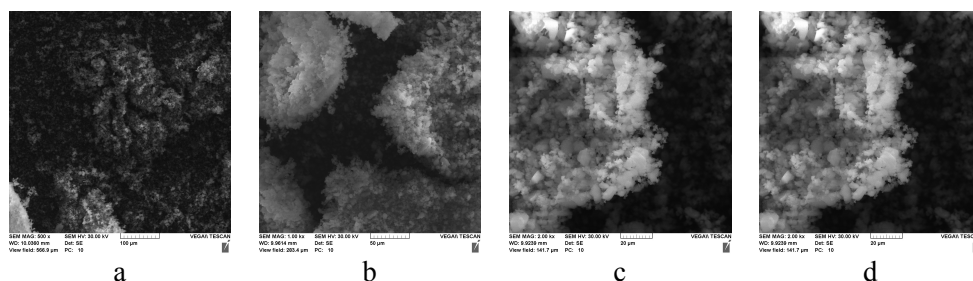


Figure 2

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

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

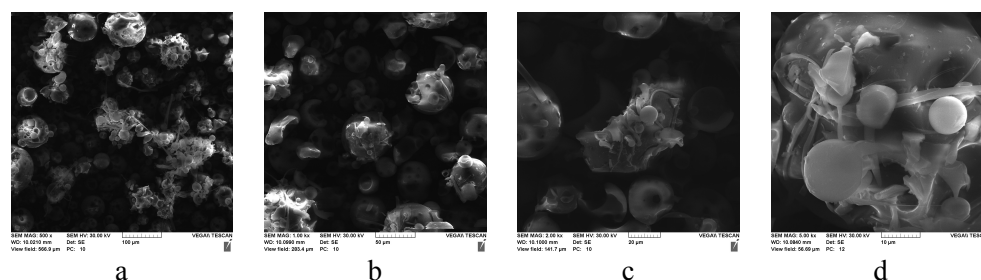
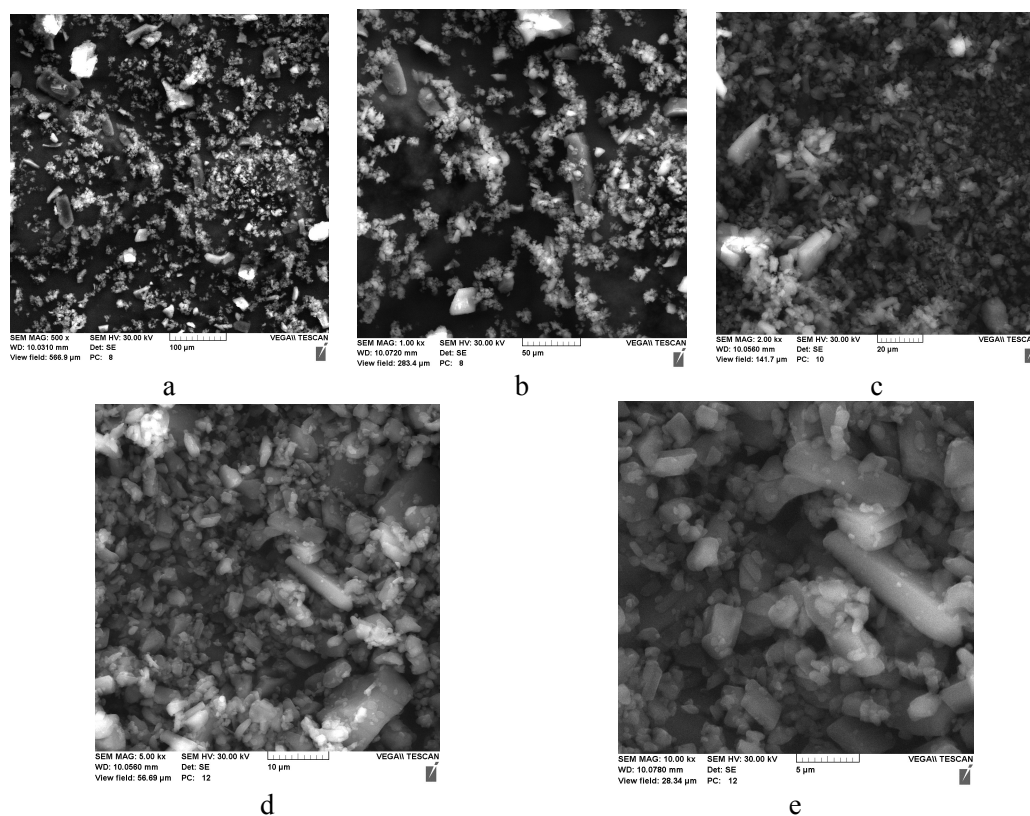


Figure 3

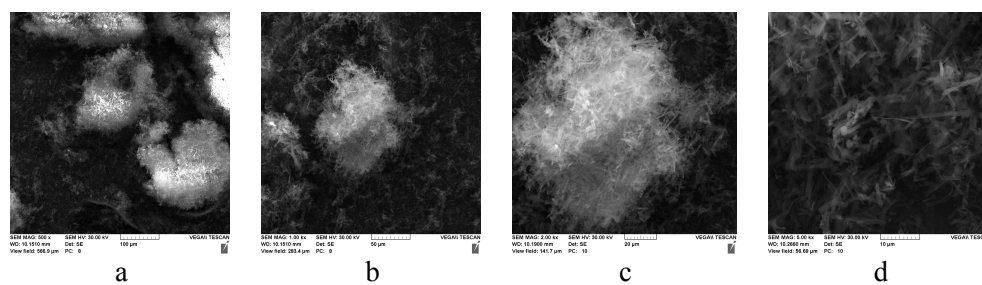
The SEM images for HP- β -CD, magnification 500x (a), 1000x (b), 2000x (c), 5000x (d)

HP- β -CD has spherical particles, with an amorphous character.

The SEM images for the SV-HP- β -CD physical mixture revealed the characteristics of SV crystals mixed with the amorphous particles of complexation agent, but the crystals of drug and the particles of CD were seen adhering to their surface, this confirming the affinity between substrates.

**Figure 4**

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

**Figure 5**

The SEM images for SV-HP- β -CD co-precipitation inclusion complex, magnification 500x (a), 1000x (b), 2000x (c), 5000x (d)

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

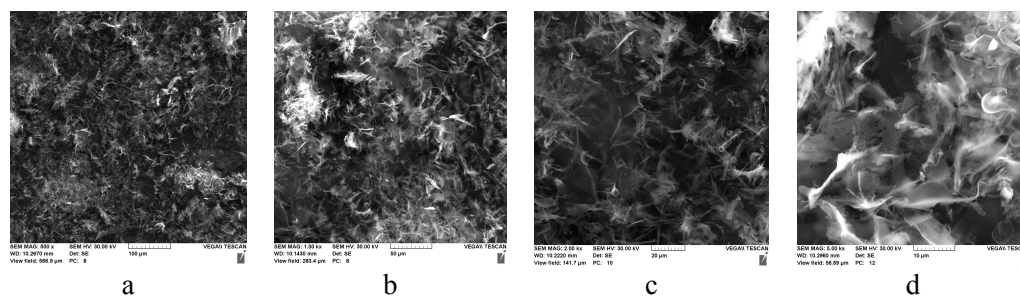


Figure 6

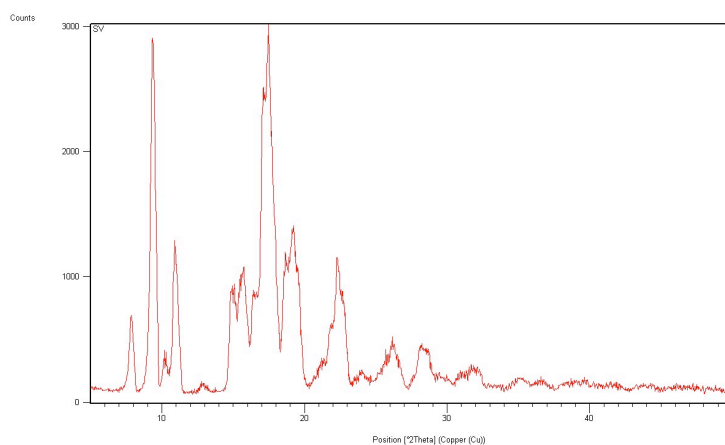
The SEM images for SV-HP- β -CD lyophilization inclusion complex, magnification 500x (a), 1000x (b), 2000x (c), 5000x (d)

In contrast with the co-precipitation inclusion complex, the lyophilization complex showed 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, SV and HP- β -CD, revealing a strong interaction between the drug and CD in these systems.

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

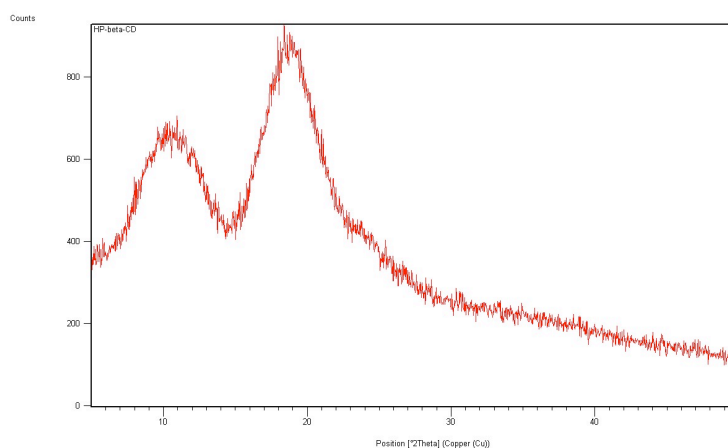
Powder X-ray diffraction (PXRD)

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

**Figure 7**

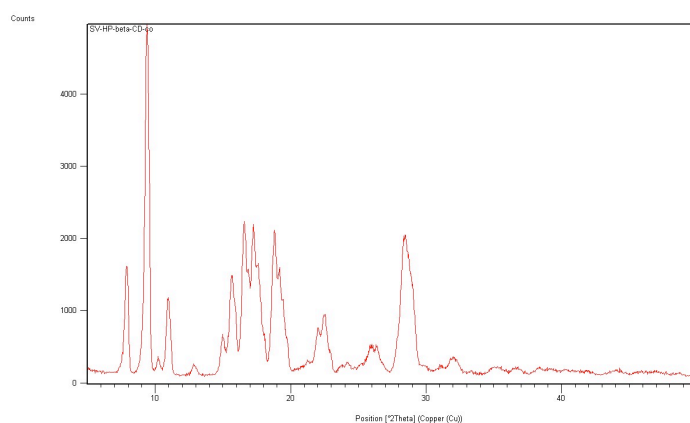
The powder X-ray diffraction pattern of SV

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° și 31.94° .

**Figure 8**

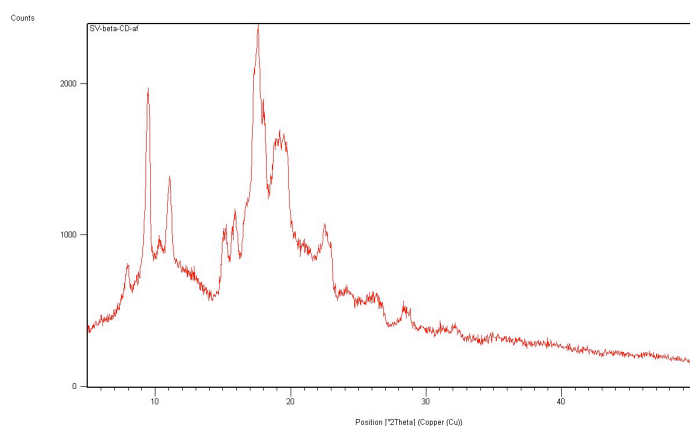
The powder X-ray diffraction pattern of HP-β-CD

On the other hand, the powder X-ray diffraction pattern of HP-β-CD confirms its amorphous form.

**Figure 9**

The powder X-ray diffraction pattern of SV - HP-β-CD physical mixture

The powder X-ray diffraction pattern of SV - HP-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 alike the one corresponding to SV, but with a decrease of peaks intensity, due to the amorphous character of HP-β-CD.

**Figure 10**

The powder X-ray diffraction pattern of SV - HP-β-CD co-precipitation inclusion complex

The powder X-ray diffraction pattern of SV - HP-β-CD co-precipitation inclusion complex showed the presence of a new compound in an amorphous form, but which maintained some small SV peaks.

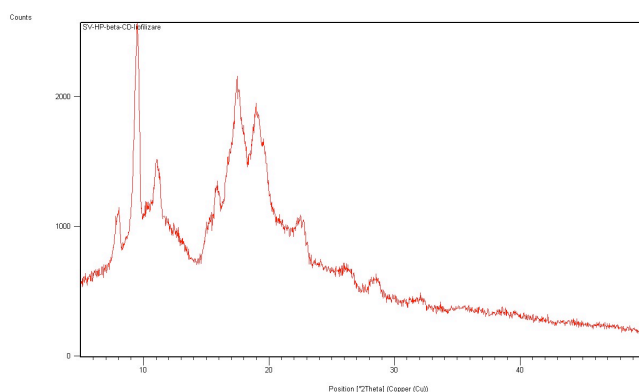


Figure 11

The powder X-ray diffraction pattern of SV - HP-β-CD lyophilization inclusion complex

The SV - HP-β-CD lyophilization inclusion complex presented the powder X-ray diffraction pattern with less peaks of low intensity, keeping somehow the basic 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) showed 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.

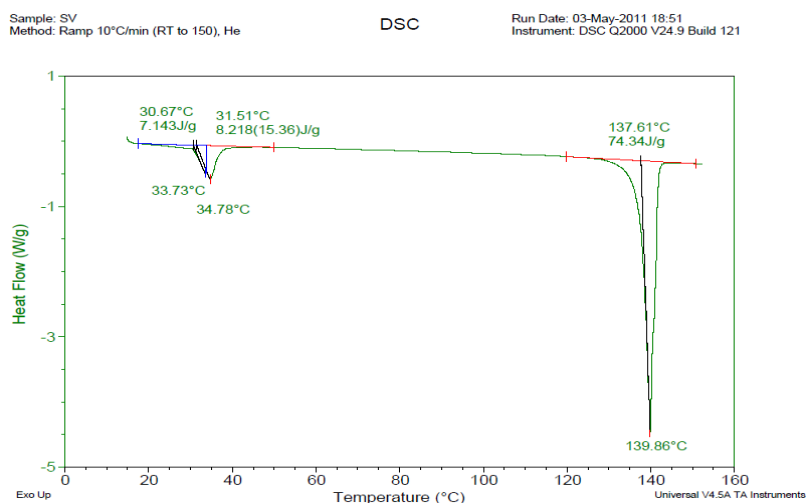


Figure 12

The DSC thermogram of SV

The DSC thermogram of HP- β -CD showed an endothermic peak at 54.83°C (figure 13), the shape of the plot being characteristic for an amorphous substance. The endothermic peaks of a low intensity appeared below 100°C, and are due to the water absorbed by the sample. [5]

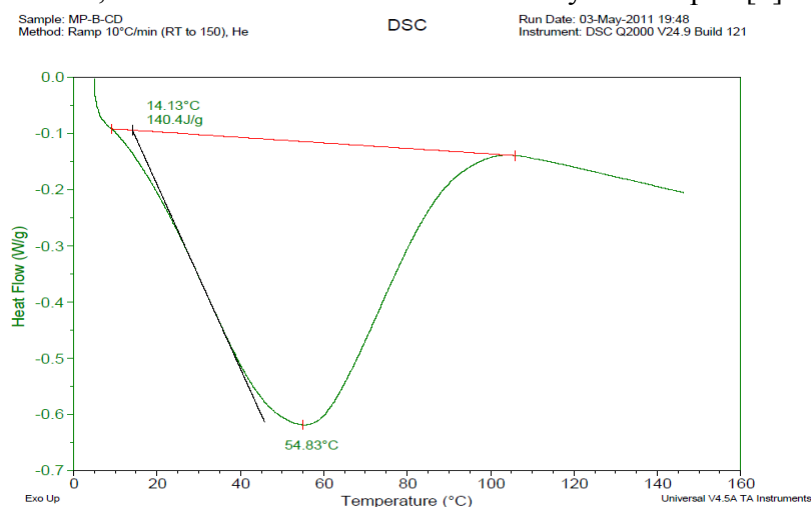


Figure 13

The DSC thermogram of HP- β -CD

The DSC thermogram of SV-HP- β -CD physical mixture (figure 14) shows two endothermic peaks, one characteristic for SV at 140.23°C, but with a low intensity, with an endothermic effect of 18.47 J/g, and the other one at 89.56°C, due to the water loss, with an endothermic effect of 294.00 J/g. This result proves that the two substances interacted, but the complexation phenomenon didn't occur.

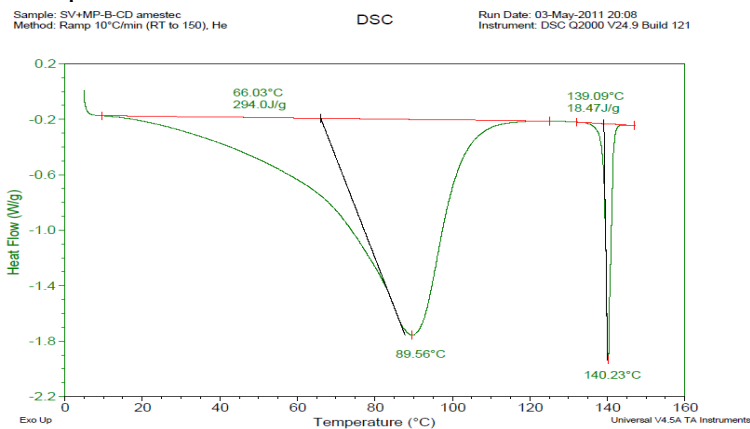


Figure 14

The DSC thermogram of SV-HP- β -CD physical mixture

For the SV-HP- β -CD co-precipitation inclusion complex, the DSC thermogram (figure 15) is characterized by the presence of only one endothermic peak at 126.34°C with an endothermic effect of 49.27 J/g similar with the one of SV, but less intense and shifted to lower temperature.

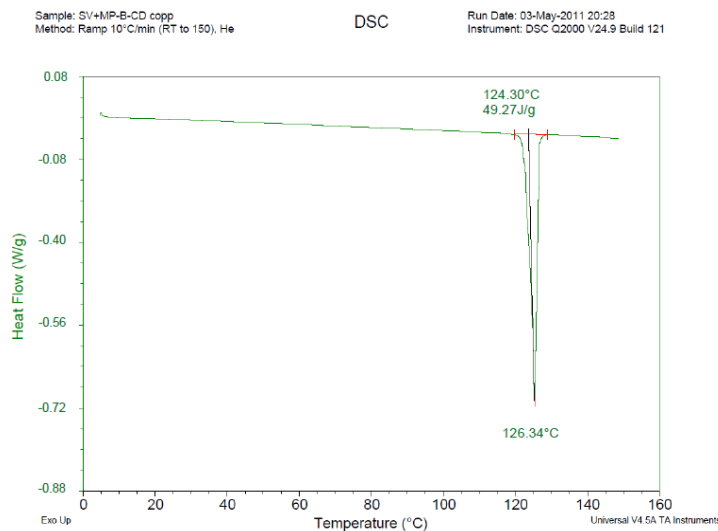


Figure 15

The DSC thermogram of SV-HP- β -CD co-precipitation inclusion complex

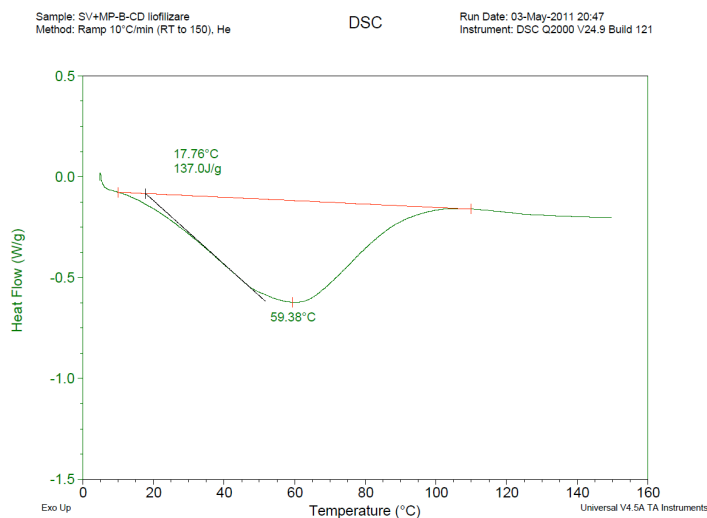


Figure 16

The DSC thermogram of SV-HP- β -CD lyophilization inclusion complex

The DSC thermograms of SV-HP- β -CD lyophilization inclusion complex (figure 16) showed only one endothermic peak at 59.38°C with a large endothermic effect of 137.00 J/g, due to the release of water molecules from the structure, being interesting the complete disappearance of the endothermic peak corresponding to SV. The DSC curve has the characteristic shape for amorphous substances, by all these proving the formation of the 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).

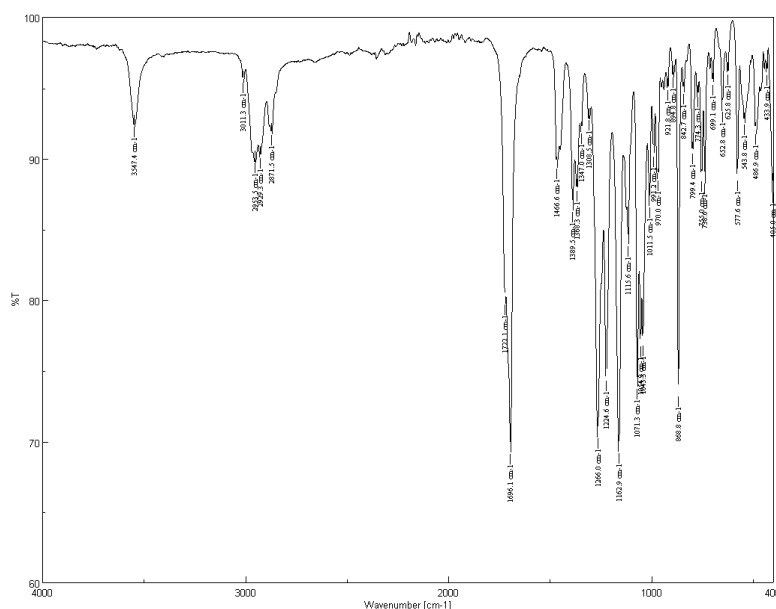


Figure 17
The FT-IR spectra of SV

The FT-IR spectra of HP- β -CD (figure 18) showed the major peaks at 3336 cm^{-1} (O–H stretching vibrations), 2970 cm^{-1} , 2926 cm^{-1} and 2884 cm^{-1} (C–H stretching vibrations) and at 1022 cm^{-1} a very strong band produced by the C–O bond vibration.

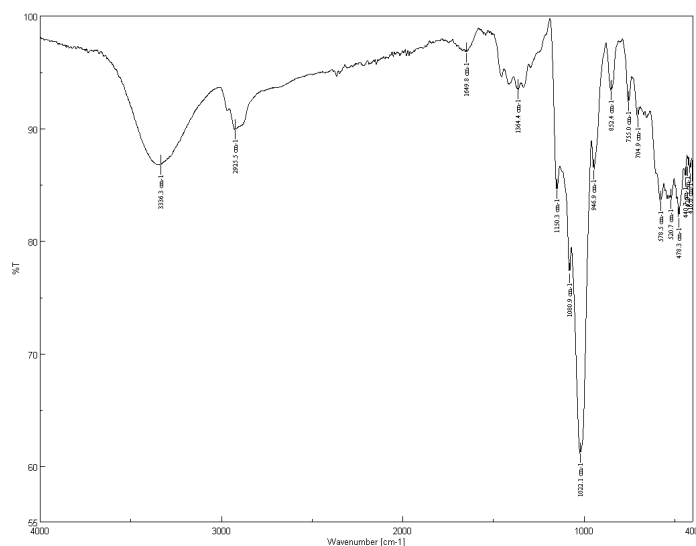


Figure 18
The FT-IR spectra of HP-β-CD

The FT-IR spectra of SV- HP-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.

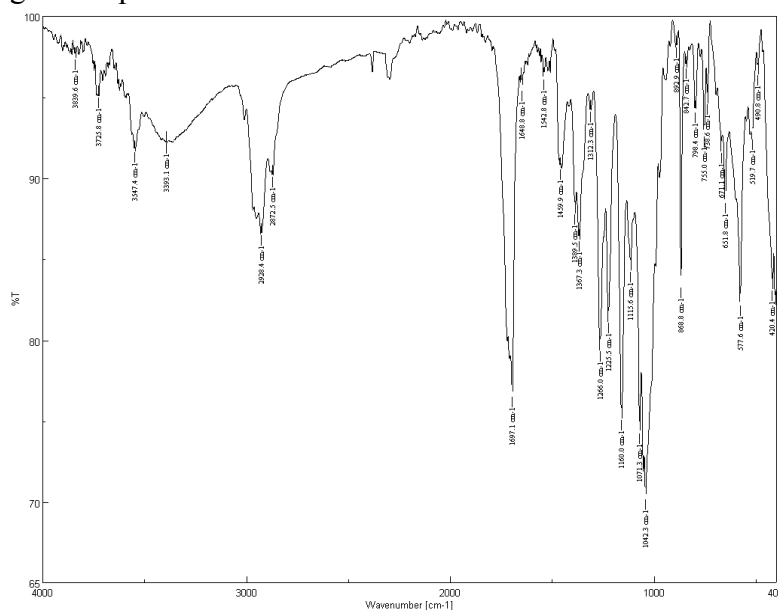


Figure 19
The FT-IR spectra of SV- HP-β-CD physical mixture

The FT-IR spectra of SV-HP- 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 1723 cm^{-1} and 1709 cm^{-1} . A higher wavelength value can also be observed for the C–O vibration (1030 cm^{-1}) and for the O–H stretching vibrations (3354 cm^{-1}), proving the formation of new weak bonds between HP- β -CD and SV in the structure of the complex.

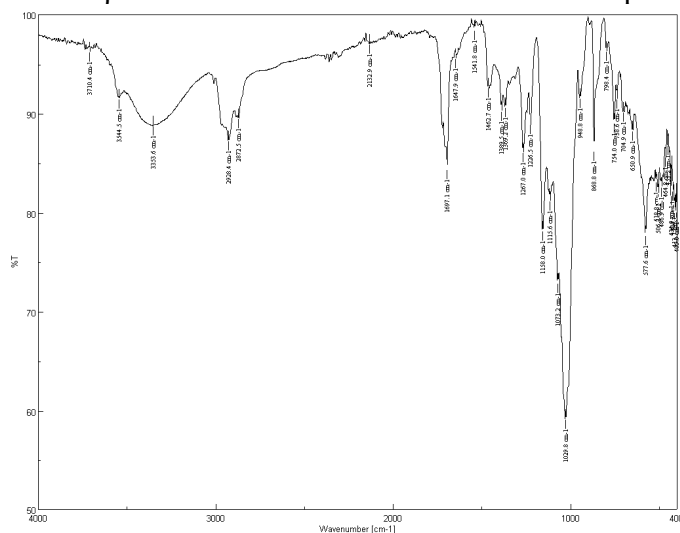


Figure 20

The FT-IR spectra of SV- HP- β -CD co-precipitation inclusion complex

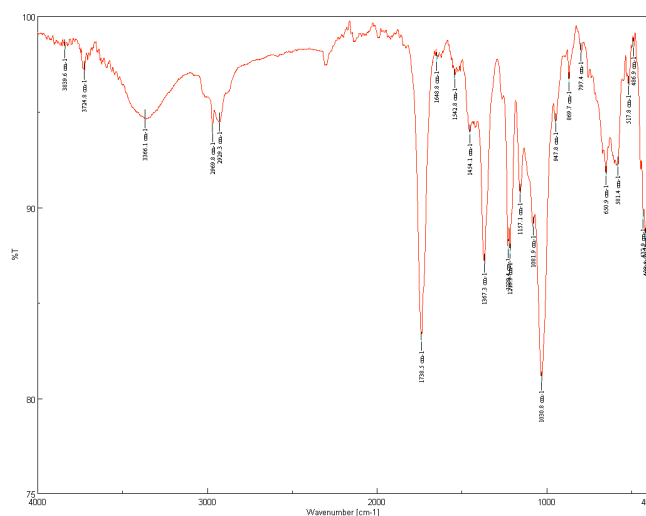


Figure 21

The FT-IR spectra of SV- HP- β -CD lyophilization inclusion complex

In the FT-IR spectra of SV-HP- β -CD liophilization inclusion complex (figure 21) the characteristic bands of the two carbonyl groups of SV have smaller transmissions (T%) in the complex and higher wavelength values, 1739 cm^{-1} and 1724 cm^{-1} . A higher wavelength value can also be observed for the C–O vibration (1031 cm^{-1}) and for the O–H stretching vibrations (3366 cm^{-1}), proving the formation of new weak bonds between HP- β -CD and SV in the structure of the complex.

Conclusions

The obtained results in the phase solubility study indicate the fact that in an aqueous solution, SV forms with HP- β -CD a soluble inclusion complex, in a molar ratio of 1:1. The phase solubility diagrams are linear, A_L type, and the value of the stability constant (K_{st}) confirms the fact that the final complex is relatively stable, and it can be concluded that HP- β -CD increases the aqueous solubility of SV.

The studied inclusion complexes between SV and HP- β -CD were obtained in a molar ratio of 1:1, by two methods, co-precipitation and lyophilization, when amorphous powders were obtained. In order to compare the results, we prepared a physical mixture between SV and HP- 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, we have noticed a remarkable change of the shape, size, and structure of the binary systems, leading us to the conclusion that the inclusion phenomenon of SV in the cavity of HP- β -CD has occurred.

The powder X-ray diffraction patterns showed that in the physical mixture there are not registered important differences compared with SV, meantime by co-precipitation and lyophilization processes, amorphous new compounds with more HP- β -CD characteristics appeared.

The DSC thermograms registered in the temperature range between 20°C and 150°C showed that by the co-precipitation method the complexation was not complete, but by the lyophilization method the complexation was complete, facts noticed by the total disappearance of SV peak corresponding to its melting point.

The FT-IR spectra revealed that the proportion in which the co-precipitation complex is formed is lower than for the lyophilization complex, proved by smaller wavelength values of the two carbonyls and for the O–H stretching vibrations.

All our studies indicated that SV forms stable and water soluble inclusion complexes with HP- β -CD in 1:1 molar ratio, the co-precipitation

method leads to a partial complexation, meantime the complexation through lyophilization is complete.

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