Colloids for controlled drug delivery:

Molecular and colloidal interactions of cyclodextrins

Doctoral (PhD) theses

András Süle

Semmelweis University
Doctoral School of Pharmaceutical and Pharmacological Sciences

Supervisor:	**Dr. Ferenc Csempesz, C.Sc. Ph.D.**
Opponents:	**Dr. Éva Fenyvesi, Ph.D.** **Prof. Dr. Sylvia Marton, Ph.D.**
Head of examination committee:	**Prof. Dr. Kornélia Tekes, C.Sc.**
Members of examination committee:	**Dr. István Pászli, C.Sc.** **Dr. György Stampf, Ph.D.**

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Introduction

Extensive studies showed that complexation of pharmacons with cyclodextrins can be a beneficial way in pharmaceutical formulations. Controlled drug delivery and targeting may enhance the efficiency and safety of medications by improving the pharmacokinetic properties of certain drug molecules.

Cyclodextrins (CDs) are extensively used complexing agents for lipophilic compounds, mainly because of their peculiar structures. Manufacturing of these biocompatible and non-toxic molecules is relatively simple and poses no threat to the environment. Their oral administration is considered to be safe, since their metabolic pathway is similar to that of starch and dextranes.

Over the past few years, a growing amount of papers pointed out that statins provide crucial benefits when used in either lipid lowering therapy or coronary heart disease (CHD) therapy. It is well documented that statins inhibit the rate-limiting steps in cholesterol synthesis. The 3-hydroxy-3-methylglutaryl-like domain of statins may attach to the appropriate binding site of the reductase enzyme therefore, sterically preventing the attachment of natural substrates. A hard obstacle in statin therapy is the extremely low aqueous solubility of most derivatives. Their bioavailability is, therefore, also low and may exhibit high variability in individuals. In addition, known food and drug interactions usually tend to increase the prevalence of statin-related side effects.

Complexation of lovastatin and simvastatin with cyclodextrins, and the effect of different additives on the formation of supramolecular complexes are in the focus of these theses. Possible ways of enhancing the aqueous solubility of the drugs in both binary and ternary systems have also been discussed.

Objectives

Interactions in aqueous solutions between statins (lovastatin and simvastatin) and cyclodextrins of different chemical structures (α-, β-, γ-CD, and methylated β-CD) were studied in the absence and the presence of dissolved small molecules and macromolecular additives, respectively. The main goal of this work was a comprehensive study of complex formation between statins and cyclodextrins in aqueous solutions, by determining the stability, possible structure and some relevant physico-chemical properties of the molecular associates.

The key subjects involved in the research program are the as follows:

- Study of the molecular interactions between the statins and the cyclodextrins in binary systems:
  - the effect of the chemical structure on the effectiveness of cyclodextrins as drug solubilizers,
  - aqueous solubility of statin-CD complexes prepared under different experimental conditions,
  - determination of the stability constants for statin-cyclodextrin complexes,
  - wetting studies by immersion enthalpy measurements on solid inclusion complexes.
- Interactions of small molecules and macromolecular additives:
  - in binary solutions with cyclodextrins,
  - with statin-cyclodextrin complexes.
- Physico-chemical characterization of binary and ternary systems by:
  - surface activity measurements,
  - NMR spectroscopy,
  - infrared spectroscopy,
  - differential scanning calorimetry (DSC),
  - circular dichroism spectroscopy.
- Physical and in vitro dissolution studies of potential model tablets made from solid complexes.

Methods

UV-vis spectrophotometric assays

Quantitative determination of α-cyclodextrin was performed according to Szejtli et al. (1978) at 503 nm wavelength using a Spektromom 195D spectrophotometer. Analytical assays for β- and γ-cyclodextrin were based on the photometric methods of Buvári and Barcza (1981).

The statin content of the solutions was determined with a UV-spectrophotometric assay based on the Ph.Hg.VIII. directives. The absorbance of lovastatin and simvastatin was measured at 236 and 240 nm, respectively. For these measurements a computer-controlled spectrophotometer (PerkinElmer Lambda Series 2S instrument) was used.

Phase-solubility measurements

The solubilising efficiency of CDs was studied according to the Higuhci-Connors procedure (1965). Various amounts of CD were generally dissolved in distilled water and lovastatin or simvastatin was added to the solutions in vast excess. In a temperature controlled procedure, twenty-four hours incubation period was ensured for the dissolution of the pharmacon at 14, 25, 36 and 47°C, respectively. After removing the non-dissolved statin from the solutions with 0.20 μm Sartorius Minisart type 16534 membrane filter, the absorption spectra of the dissolved pharmacon were then taken in the range of 190-400 nm wavelengths.

The solubility of statins was also investigated at two moderate pH values, relevant to that of the gastro-intestinal tract. To adjust the pH of the solutions, buffers prepared on the basis of the directives of the Ph.Hg.VIII. were used ($pH_{gastric}$=1.2; $pH_{intestinal}$=7.4).

For the preparation of statin-CD-polymer ternary systems, a specially controlled thermal program was used. The appropriate mixtures were heated up to 70°C and kept at this elevated temperature for 2 hours. After that, the solutions were cooled down to 25°C and kept at this temperature for 22 hours.

Surface tension determination

The surface tension of aqueous solutions was measured by computer controlled Wilhelmy-plate method, using a KSV Sigma 70 instrument. The measurements were

carried out at 25°C and the experimental data were evaluated by the Origin 7.0 software suite.

NMR spectroscopy

NMR spectra of the CDs, the native statins and of the statin-CD complexes were taken using a Varian Unity Inova type 600 instrument in deuterated water (D_2O) and methanol (CD_3OD), respectively.

Immersion enthalpy measurements

Wetting studies on solid complexes were carried out in 50 ml distilled water, using a Setaram-Calvet type MS-70 microcalorimeter. The immersion enthalpy values(Q_w) were calculated from the registered heat intensity vs. time plots.

Differential scanning calorimetry (DSC)

A PerkinElmer DSC-II instrument was used for DSC-measurements. The spectra were taken in a wide temperature range from 25°C to 250°C at a heating rate of 10°C/min.

Infrared spectroscopy

Infrared spectra were taken using Fourier-transformed attenuated total reflection (ATR-FTIR) technique in a PerkinElmer 1650 instrument. Prior to the measurements, acetonic solutions or suspensions were dried on the horizontal ATR probe.

In vitro dissolution studies of potential model tablets

Drug release and dissolution studies were carried out in 500 ml distilled water at 37 ± 0.5°C using a Pharma Test type PTW-II instrument running at 50 rpm speed. Concentrations of the dissolved pharmacon were determined by UV-vis spectroscopy.

Results and conclusions

1. Dissolved statin-cyclodextrin complexes

1.1. Molecular interactions between dissolved statins (lovastatin, simvastatin) and cyclodextrins were shown by phase-solubility measurements *(Figure 1.)*.

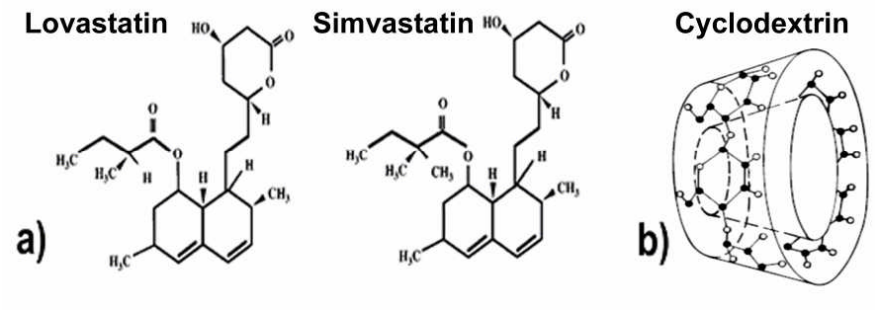

Figure 1. – a., Chemical structure of lovastatin and simvastatin
b., Molecular structure of α-cyclodextrin

1.2. It was found that at physiologically relevant temperature and pH levels the solubility of the studied statin derivatives can be improved by 1 or 2 orders of magnitude *(Figure 2.).*

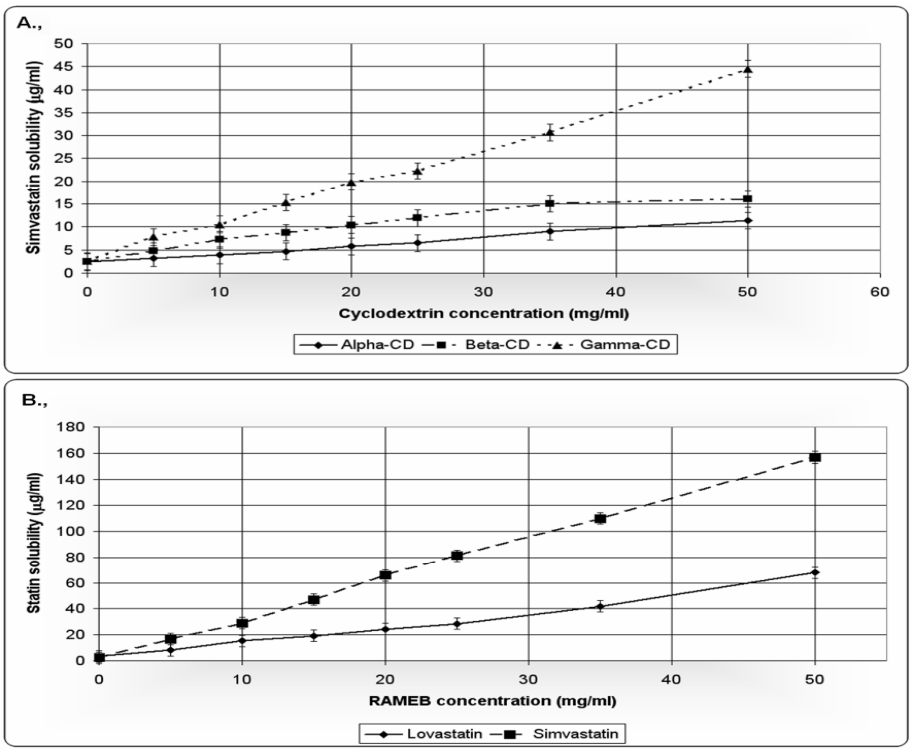

Figure 2. – Phase-solubility curves of lovastatin and simvastatin in CD-solutions, T=25°C, pH≈6,5

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1.3. Stability constants for the associates of different molar ratios have been calculated by two independent calculation procedures. The corresponding association constant are in good correlation *(Table 1)*.

Table 1. – Stepwise stability constants of statin-CD complexes,,
K^*_{11} – values determined from the solubility isotherms
K_{11}, K_{12} – values calculated by using an iteration method

	Lovastatin - βCD	**Lovastatin - RAMEB**	**Simvastatin - βCD**	**Simvastatin - RAMEB**
K*11 ***(isotherm)***	$1.9 \cdot 10^2$	$2.1 \cdot 10^2$	$1.5 \cdot 10^3$	$1.7 \cdot 10^3$
K11 ***(iteration)***	$1.86 \cdot 10^2$	$9.38 \cdot 10^2$	$2.88 \cdot 10^2$	$1.21 \cdot 10^3$
K12 ***(iteration)***	$1.13 \cdot 10^2$	$1.61 \cdot 10^2$	$2.63 \cdot 10^1$	$2.38 \cdot 10^2$

1.4. The molecular structure of statin-CD complexes was established from the results of ^{1}H-NMR, ^{13}C-NMR measurements using 2D-ROESY and DOSY methods. The carbonyl-chains of simvastatin show intermolecular spatial proximity with H3 and H5 hydrogens of the cyclodextrins' inner cavities. These results prove the existence of host-guest inclusion complexes of 1:1 molar ratios *(Figure 3.)*.

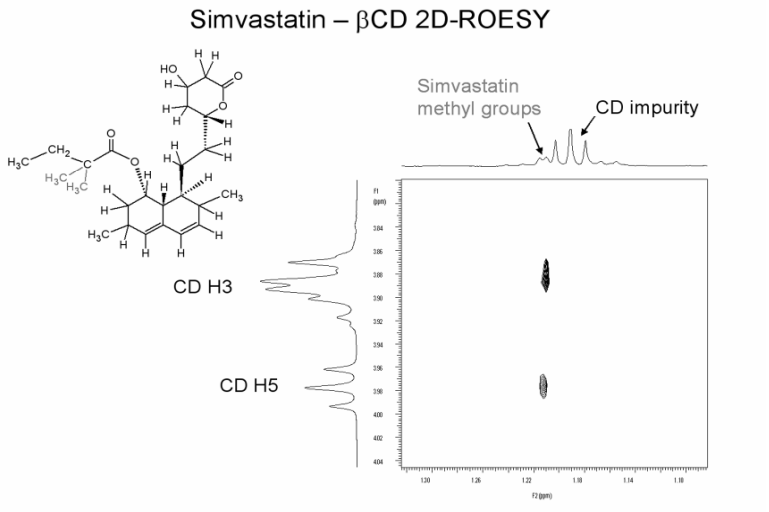

Figure 3. – 2D-ROESY spectrum of simvastatin – βCD complexes

1.5. In the presence of suitable macromolecular colloid (polyvinyl pyrrolidone, PVP), the solubility of both statins can further be improved *(Figure 4/a.)*. On the contrary, drug solubility was notably reduced by the chemically analogous monomer compounds (N-ethyl-pyrrolidone, N-methyl-pyrrolidone, NEP, NMP) of the polymer additive,

presumably due to a competition between the molecules of the drug and the small molecular weight additive *(Figure 4/b.)*. The apparent increase of drug solubilities in polymer-containing systems can possibly be attributed to the formation of supramolecular ternary complexes.

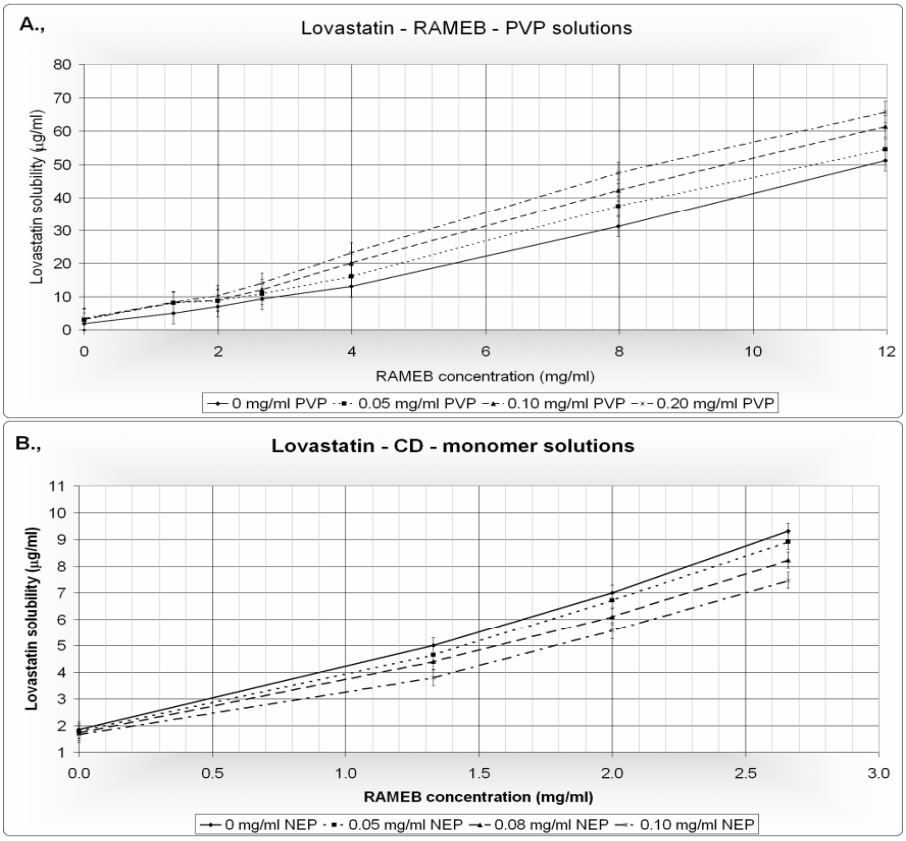

Figure 4. – a., Effect of PVP on the solubility of lovastatin in RAMEB solutions
b., Effect of NEP on the solubility of lovastatin in RAMEB solutions

1.6. Inclusion of NMP molecules in the cyclodextrin cavities in ternary systems was shown. These results provide solid experimental background to the assumed competitive mechanism. 2D-ROESY spectra showed no intermolecular spatial proximity between PVP segments and the cyclodextrin cavity, which also suggests the formation of supramolecular ternary complexes in polymer-containing systems.

1.7. Considerable surface activity of the dissolved components in statin-CD solutions was demonstrated. It can be concluded that formation of statin-CD complexes leads to the development of surface-active solutes, which can accumulate at the air/solution interface. *(Figure 5.)*.

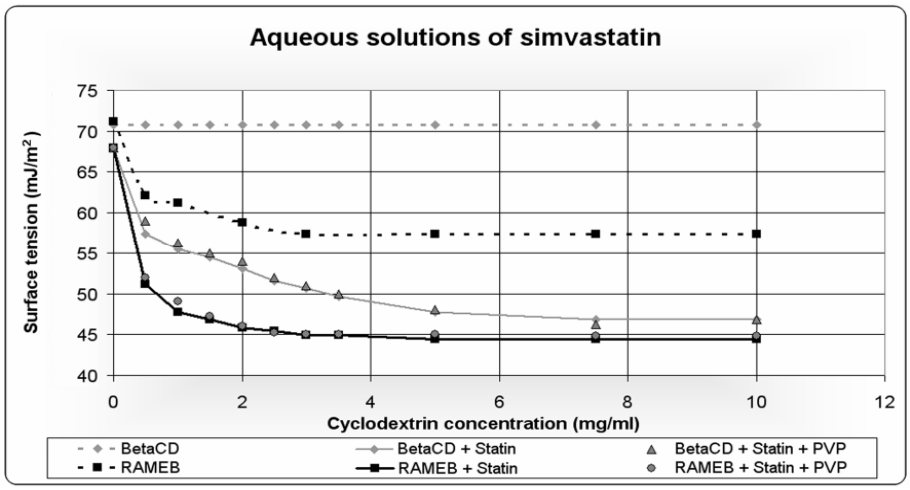

Figure 5. – Change of the surface tension in CD-containg solutions

Complexation of statins can also be regarded as a CD-induced "amphiphilization" of the hydrophobic drug molecules. The enhanced drug solubilities in polymer solutions can be likely ascribed to the formation of ternary associates. The surface-active complexes may attach to the dissolved macromolecules in a way that they are anchored by their hydrophobic part at the polymeric chains.

1.8. Certain compounds of small molecular mass additives also resulted in further increase in the solubility of statin-cyclodextrin complexes. The additives (hydroxy acids, urea, etc.) that can disrupt the hydrogen-bond system of cyclodextrins in aqueous solutions might improve the efficiency of solubilisation *(Figure 6/a-b.)*.

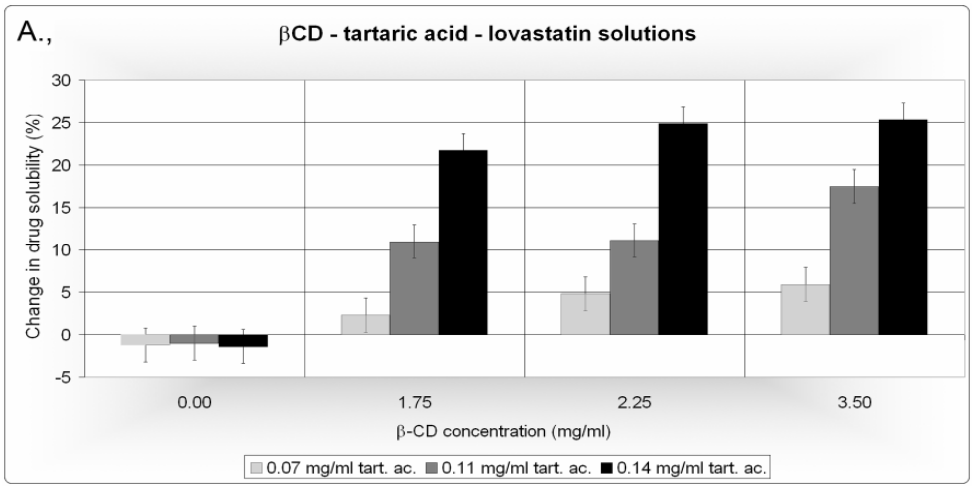

Figure 6. – a., Effect of tartaric acid on lovastatin solubility in β-CD solutions, T=25°C, pH≈6,5

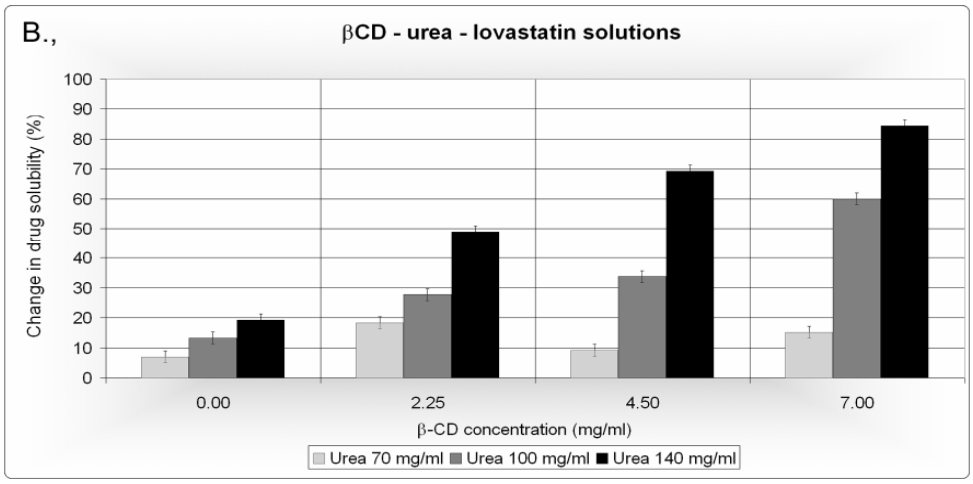

Figure 6. – b., Effect of urea on lovastatin solubility in β-CD solutions, T=25°C, pH≈6.5

1.9. Circular dichroism spectroscopy and conductivity studies provided no evidence of selective drug complexation of cyclodextrins. Preference for hydroxy-acidic statin forms in complexation could not be detected. The hydrolysis equilibrium of aqueous statin-CD solutions remained unaltered in these systems.

2. Solid statin-cyclodextrin complexes

2.1. Immersion enthalpy values (Q_w) of statin-CD complexes determined by microcalorimetry were 3-fold higher than that of the native cyclodextrins, and 5-fold higher than the immersion enthalpy of the pharmacons.

2.2. In accordance with the spectroscopic studies, infrared spectra of solid complexes indicated that the characteristic absorption peak of water in pure βCD disappeared from the spectrum of the statin-CD complexes. Moreover, as a result of complexation, peaks corresponding to the primary and secondary hydroxyl-groups of CDs get shifted, providing an indirect proof of the inclusion mechanism.

2.3. DSC measurements confirmed that the inclusion complexes have significantly different physical structures compared to that of the individual components. The characteristic peak of simvastatin disappears from the spectrum of the statin-CD complexes, suggesting an amorphous character of the pharmacon molecules *(Figure 7.)*.

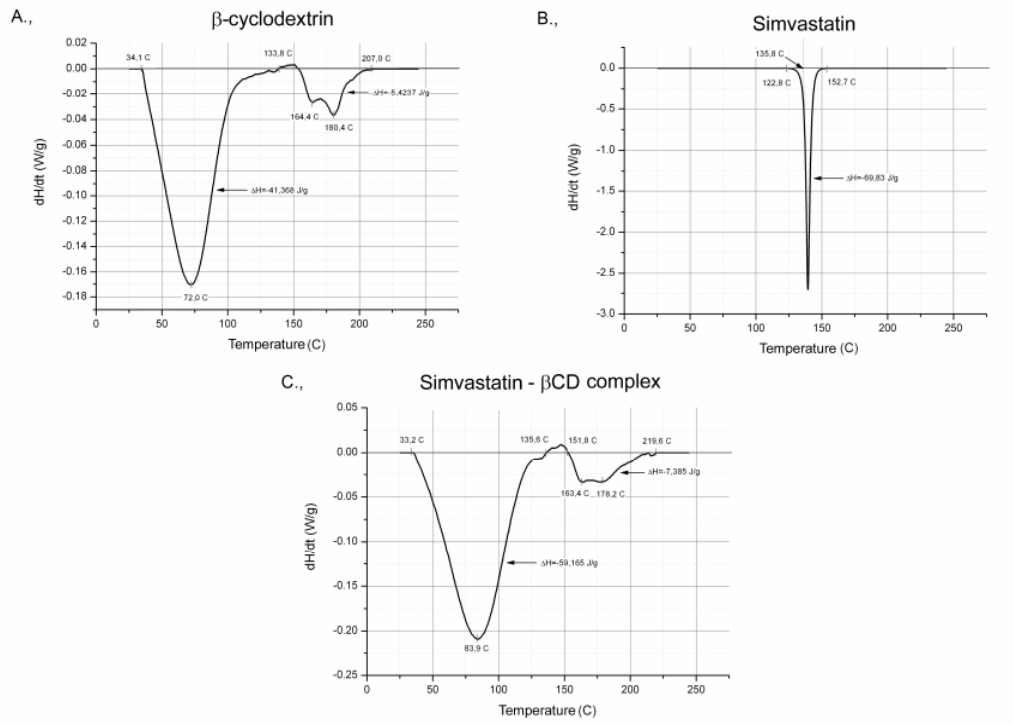

Figure 7. – a., DSC-curve of β-cyclodextrin
b., DSC-curve of simvastatin
c., DSC-curve of simvastatin – βCD complexes

2.4. Preliminary in vitro dissolution studies performed with potential model tablets and effervescent tablets, respectively showed that complexation with cyclodextrins can be successfully used for enhancing the release and dissolution of statins and for improving the physical structure of the tablets.

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