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Use of cyclodextrins as solubilizing agents for simvastatin: Effect of hydroxypropyl-β-cyclodextrin on lactone/hydroxyacid aqueous equilibrium

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ABSTRACT

The chemical conversion of simvastatin from the lactone (SVL) to the hydroxyacid (SVA) form is becoming an intriguing issue associated with the pharmacological use of SVL. On this matter, recent findings suggest that SVL complexation with cyclodextrins (CDs) may be a useful strategy to affect its aqueous solubility and chemical stability. In this work, a reverse-phase high-performance liquid chromatography (RP-HPLC) method able to selectively identify and quantify SVL and SVA has been set up, validated and applied to follow SVL hydrolysis in the presence of HPβCD. The combination of stability results with simvastatin/HPβCD stability constants achieved from UV–vis measurements and solubility/dissolution studies allowed to get an insight into SVL/HPβCD, SVA/HPβCD and SVL/SVA equilibria taking place in aqueous solution. Results show that in the presence of HPβCD the aqueous SVL/SVA equilibrium is shifted *versus* the hydroxyacid form. UV–vis results, showing that the lactone and the open-ring form of simvastatin interact with HPβCD in a similar extent, suggest that hydrolysis occurs also on SVL/HPβCD complex, thus supporting a mode of interaction that does not involve the lactone ring. This hypothesis is strengthened by NMR analysis performed on SVA, HPβCD and their inclusion complex, which indicates that the lactone ring is not included in HPβCD hydrophobic cavity. Finally, results suggest that particular attention must be paid to SVL lactonization in aqueous solution when using CD-based formulations and in demonstrating their effective benefit for a specific therapeutic use.

1. Introduction

Simvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase belonging to the class of statins (Schachter, 2005). Lactone ring of simvastatin undergoes reversible hydrolysis at a rate which is usually pH-dependent (Kaufman, 1990; Kearney et al., 1993; Alvarez-Lueje et al., 2005). At physiological and higher pH values, the lactone form of simvastatin (SVL) is unstable and the equilibrium favours hydrolysis opening the lactone and yielding the hydroxyacid form (SVA) (Fig. 1) (Alvarez-Lueje et al., 2005). Under acidic conditions, SVA is susceptible to lactonization to give again SVL. In general, SVL and SVA co-exist in equilibrium *in vivo*, and often the statin lactone form is at least as abundant as the hydroxy acid.

SVL is a poorly water soluble inactive prodrug that undergoes enzymatic and chemical conversion in the intestine, plasma, and liver to SVA, the main pharmacologically active metabolite. After conversion, SVA acts by decreasing cholesterol synthesis and increasing low density lipoprotein (LDL) catabolism, that turns in the reduction of cholesterol levels and subsequent prevention of coronary heart diseases (Todd and Goa, 1990; Schachter, 2005). Since cholesterol synthesis occurs mainly in the liver, the main advantage of administering SVL rather than SVA is its high selectivity for distribution into the liver. Furthermore, SVA, but not SVL, is substrate of the P-glycoprotein efflux transporter (Hochman et al., 2004; Chen et al., 2005), which can reduce its intestinal absorption and enhance the elimination of its substrates (Leschziner et al., 2007).

Because of extensive first-pass metabolism, the bioavailability of oral simvastatin is still less than 5% and several strategies aimed at modifying release rate of either the prodrug (i.e., SVL) or the active moiety (i.e., SVA), are under development to increase oral bioavailability (Vickers et al., 1990; Neuvonen et al., 2006). Meanwhile, recent clinical evidences suggest that statins have additional pharmacological properties such as endothelial protection *via* intervention in the nitric oxide synthase system as well as antioxidant and anti-inflammatory effects (Endres, 2006; Gelosa et al., 2007; Martinez-Gonzalez and Badimon, 2007). Of particular interest is the anabolic effect of SVA, not SVL, on bone, again due to its interference on the mevalonate pathway (Garrett et al.,

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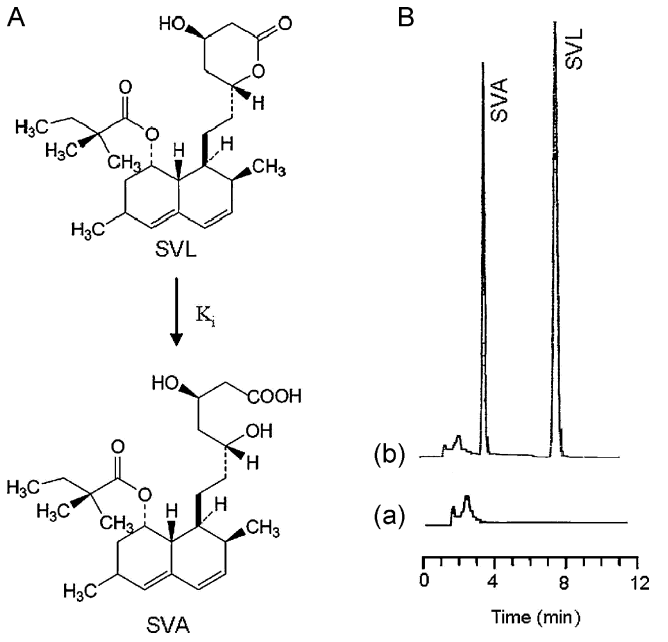

Fig. 1. Chemical conversion of simvastatin lactone (SVL) into simvastatin hydroxyacid (SVA) governed by the hydrolysis constant K_h (A). A representative chromatogram relative to RP-HPLC analysis of a standard mixture of SVL (t_r = 7.2 min) and SVA (t_r = 4.2 min) is shown (B). A RP-HPLC chromatogram of the solvent (t_o = 2.3 min) is reported for comparison.

2001; Bauer, 2003). For these extension applications it is crucial that the right form of simvastatin (i.e., SVL or SVA) is expressed at the action site with the aim to optimize pharmacological effect. In this sense, the chemical equilibrium SVL/SVA becomes an intriguing issue and its control by acting on pharmaceutical composition a valuable strategy.

Cyclodextrins (CDs) are well known molecular entities used as pharmaceutical excipients mainly to solubilize and stabilize drugs (Loftsson and Brewster, 1996). Guest/CD complexes offer a variety of physicochemical advantages over the unmanipulated drugs including the possibility for increased water solubility and drug stability (Loftsson and Duchene, 2007; Brewster and Loftsson, 2007). Results of previous studies demonstrated that in aqueous media a host–guest type inclusion complexation of lovastatin and simvastatin lactones with several CDs (α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and RAMEB) takes place and possibly enhances the aqueous solubility of the drugs (Sule et al., 2009). Taking advantages of this effect, SVL/hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complexes orally administered to rats displayed a higher hypolipidemic activity – in term of total cholesterol serum levels – as compared to free SVL (Jun et al., 2007). On the other hand, SVA/β-cyclodextrin interactions were proven to be useful also in controlling the selective release of SVA from coatings of titanium plates intended for local administration of the drug (Yoshinari et al., 2007). However, in all of these studies, the effect of CD on the SVL/SVA equilibrium in aqueous solution was not taken into account.

The aim of this work is to highlight that a traditional preformulation study on CD-based simvastatin dosage forms cannot neglect the contribution of HPβCD on SVL/SVA conversion. To this end, a reverse-phase high-performance liquid chromatography (RP-HPLC) method able to selectively identify and quantify SVL and SVA has been set up, validated and applied to follow simvastatin hydrolysis in the presence of HPβCD. The combination of stability results with solubility and dissolution studies of simvastatin in the presence of HPβCD allowed to get an insight into SVL/SVA equilibrium taking place in aqueous solution highlighting possible pharmaceutical implications.

2. Experimental

2.1. Materials

Simvastatin lactone (SVL) and hydroxypropyl-β-cyclodextrin (Mw 14,598 Da, molar substitution 0.8) (HPβCD) were purchased from Sigma–Aldrich (USA). Analytical grade potassium phosphate monobasic, sodium hydroxide, ethanol, dichloromethane, and HPLC grade acetonitrile were supplied by Carlo Erba (Italy).

2.2. Preparation of simvastatin-β-hydroxyacid

SVL was hydrolyzed to its β-hydroxyacid form and isolated as open-ring SVA (Yoshinari et al., 2007). Briefly, 1.5 mL of sodium hydroxide (NaOH 0.1 N) were added to 1 mL of a SVL solution in ethanol (40 g/L). The resulting solution was incubated in a stove (Vuototest, Mazzali, Italy) at 50 °C and 1 atm for 2 h to open up the lactone ring. Residual ethanol was allowed to evaporate under stirring overnight. Afterward, SVA solution was freeze-dried for 24 h at 0.01 atm and −60 °C in a Modulyo apparatus (Edwards, UK). The resulting dry powder was dispersed in acetone to precipitate any residual NaOH, and centrifuged at 5000 rpm for 15 min (Hettich Zentrifugen, Universal 16R). The supernatant was collected and dried by rotary evaporation at 40 °C and 30 rpm (Laborota model 4010, Heidolph, Germany). The film obtained was treated with water and the sample freeze-dried for 24 h at 0.01 atm and −60 °C in a Modulyo apparatus (Edwards, UK). SVA was identified by electrospray ionization mass spectrometry (ThermoFinnigan LCQ Ion-Trap) analysis performed on the product eluted by RP-HPLC as described in the following paragraph.

2.3. Simvastatin HPLC analysis

SVL analysis was carried out by RP-HPLC on a system consisting of a FCV-10 ALvp mixer and a LC-10ADvp pump equipped with a SIL-10ADvp autoinjector, a SPD-10Avp UV–vis detector and a C-R6 integrator from Shimadzu (Japan). The analysis was performed at 25 °C on a Sinergy-Max RP C12 column (150 mm × 4.6 mm, 300 Å) (Phenomenex, USA). The mobile phase was a 30:70 (v/v) mixture of 0.05 mM phosphate buffer at pH 3.0 and acetonitrile pumped at a flow rate of 1.0 mL/min. The injection volume was 20 μL in all the experiments and the detection wavelength fixed at 238 nm.

Standard solutions of either SVL or SVA were prepared in ethanol and stored at 4 °C in the dark until use. Calibration curves were obtained by injecting solutions at 0.24, 0.48, 0.96, 1.2, 2.4, 4.8, 12.0 and 24.0 μg/mL. Ethanol solutions of SVL or SVA were stable at least for one week.

2.4. Complexation of simvastatin with HPβCD

The variation of drug UV–vis spectrum in the presence of HPβCD was measured to determine the stability constant of SVL/HPβCD and SVA/HPβCD complexes. To this end, stock solutions of either SVL or SVA at the concentration of 0.2 mg/mL in ethanol were prepared. Triplicate 5.0 mL samples, each containing 8 μg/mL of the native drug, were obtained by diluting aliquots of 0.2 mL of the stock solution with water (control) or the appropriate HPβCD aqueous solution in glass flasks (final HPβCD concentration within the range 2.0×10^{-4} to 8.2×10^{-3} M). UV–vis absorption spectra of the solutions were immediately recorded on a Shimadzu UV-1204 (Japan) spectrophotometer fitted out with 1-cm quartz cell. Data were processed by the double reciprocal method according to the

Scott's equation (Scott, 1956):

$$\frac{[HP\beta CD]\cdot[Dr]}{\Delta Abs} = \frac{1}{k_f \Delta_\varepsilon} + \frac{[HP\beta CD]}{\Delta_\varepsilon} \quad (1)$$

where ΔAbs is the difference in absorbance at 238 nm between the complexed and free drug, Δ_ε is their difference in the molar absorptivities, [Dr] is drug molar concentration and [HPβCD] is the molar concentration of uncomplexed HPβCD (assumed to correspond to the total amount of HPβCD added). Data were fitted by linear regression analysis according to Eq. (1) by an Excel software package. The apparent stability constant k_f was calculated from the intercept/slope ratio supposing the formation of a complex with a 1:1 stoichiometry.

Mode of interaction of SVA with HPβCD was evaluated by NMR. Spectra were acquired on a Bruker Avance instrument at ^{1}H operating frequency of 500 MHz. The samples were prepared by dissolving suitable amounts of SVA, HPβCD and SVA/HPβCD at 1:1 mole ratio in 1 mL of D_2O. The spectra were collected at 305 K and with residual HOD signal presaturation.

2.5. Simvastatin lactone/β-hydroxyacid form equilibrium in aqueous solution

A stock solution containing 0.2 mg/mL of SVL in ethanol was prepared. Triplicate 20 mL samples, each containing 8 μg/mL of the native drug, were obtained by diluting aliquots of 0.8 mL of the stock solution by water (control) or the appropriate HPβCD aqueous solution (1:5 SVL/HPβCD mole ratio) in glass flasks. The flasks were placed in a thermostatic agitating bath at 37 ± 1 °C in the dark. At scheduled time intervals, 1 mL of the medium was withdrawn and analysed for SVL and SVA content by RP-HPLC as described above. In all trials, the pH of each sample was periodically measured and did not vary by >0.1 unit. Data analysis was performed applying a pseudo first-order, reversible kinetic model describing lactone hydrolysis to the hydroxy acid form (Kaufman, 1990):

$$C_t = (C_0 - C_e)e^{-K_h^t} + C_e \quad (2)$$

where C_0, C_e, and C_t are the initial, equilibrium and time t concentrations of SVL (μg/mL), t is the incubation time (hours), k_h is the observed rate constant for SVL hydrolysis (h^{-1}). Data were fitted by regression analysis according to Eq. (2).

2.6. Solubility study

Equilibrium solubility of SVL in the presence of HPβCD was evaluated according to the method of Higuchi and Connors (Higuchi and Connors, 1965). An excess of SVL (5 mg) was added to 5 mL of water containing increasing amounts of HPβCD (0 to 1.1×10^{-2} M) and shaken in screw capped glass vials at 25 °C until equilibrium (achieved after four days). A 50 μL aliquot was withdrawn, filtered (HA 0.22 μm-filters, Millipore, USA) and analysed for SVL and SVA content by RP-HPLC as reported above. Binding constants ($k_{1:1}$) were calculated from the linear graph obtained by plotting the concentration (mM) of total simvastatin (i.e., SVL *plus* SVA) or SVL in the solution *versus* HPβCD concentration (mM) according to the equation:

$$k_{1:1} = \frac{\text{slope}}{[\text{intercept}\cdot(1-\text{slope})]} \quad (3)$$

Solubility data were fitted by linear regression analysis by an Excel software package.

Table 1
Chromatographic parameters and precision data for SVL and SVA RP-HPLC analysis (CL 95%; $n = 21$).

Parameter	Drug	
	SVL	SVA
Retention factor (k')	2.1	0.83
Selectivity (α)	2.6	
Concentration range (μg mL^{-1})	0.24–24.0	0.24–24.0
LOD (ppb)	7.1	3.4
QOD (ppb)	23.5	11.3
Regression equation (y)[a]		
Slope (a)	25435	21055
Intercept (b)	1162	2586
Correlation coefficient (r^2)	0.9926	0.9984
RSD[b]	1.98	0.93
F[c]	2555	11513

[a] $y = ax + b$, where x is the concentration in μg mL^{-1}.
[b] Relative standard deviation (%).
[c] F test for significance was applied to the regression.

2.7. Preparation and characterization of simvastatin/HPβCD binary systems

A Simvastatin/HPβCD inclusion complex at 1/1 (mol/mol) stoichiometric ratio was prepared by kneading method and spray drying. For kneading (KN), native simvastatin and HPβCD powders were placed in an agate mortar, added with 20 μL of a water/ethanol solution (50/50, v/v) and mixed by hand. The resulting mixture was dried overnight in a vacuum stove (Vuototest, Mazzali, Italy) at 41° C. For spray drying (SD), 30 mL of a simvastatin (700 mg)/HPβCD solution in ethanol/methylene chloride mixture (2/1, v/v) (Mini Spray-Dryer Büchi 190, Flawil, Switzerland). The solution was spray-dried with the following process parameters: feed rate 2 mL/min; aspirator setting 20; spray-flow 600 Nl/h; inlet temperature 115 °C. A 0.5 mm nozzle was used throughout the experiments. Powders were collected and dried for 24 h.

Crystallinity of SVL/HPβCD binary systems was assessed on a differential scanning calorimeter equipped with an intracooler (DSC 822e, Mettler-Toledo, Switzerland). Indium/Zinc standards were used to calibrate the DSC temperature and enthalpy scale. The samples were hermetically sealed in aluminum pans and heated at a constant rate of 5 °C/min over a temperature range of 20–180 °C. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50 mL/min.

Dissolution profiles of simvastatin and its complexes with HPβCD were evaluated according to USP, apparatus 2 method, in a Sotax AT7 system (Sotax, Italy). Powders (amount equivalent to 10 mg of simvastatin) were placed in 0.5 L of PBS at pH 7.4 and 37.0 ± 0.1 °C, with a paddle rotation speed of 30 rpm. The results are reported as SVL *plus* SVA dissolved (mg) ± SD of three replicates.

3. Results

3.1. Simultaneous quantitation of simvastatin lactone and simvastatin β-hydroxyacid by RP-HPLC

A HPLC method able to selectively identify and quantify simvastatin in its lactone and hydroxyacid forms has been set up (Table 1). The retention or capacity factor (k') of native simvastatin (SVL) was 2.1, whereas it was 0.83 for its β-hydroxyacid form (SVA) (Fig. 1), confirming the good selectivity of the proposed method ($\alpha \gg 1.2$). Data error-plot along with residual (weighed residuals percent) plot have been reported in Fig. 2. As can be seen, a linear relationship between chromatographic peak areas and amounts of drug was found over the concentration range 0.24–24.0 μg/mL, and $r^2 > 0.99$ was obtained in each case. The detection limit (LOD) (estimated as three times the background noise) was 7.1 μg/L for SVL and 3.4 μg/L

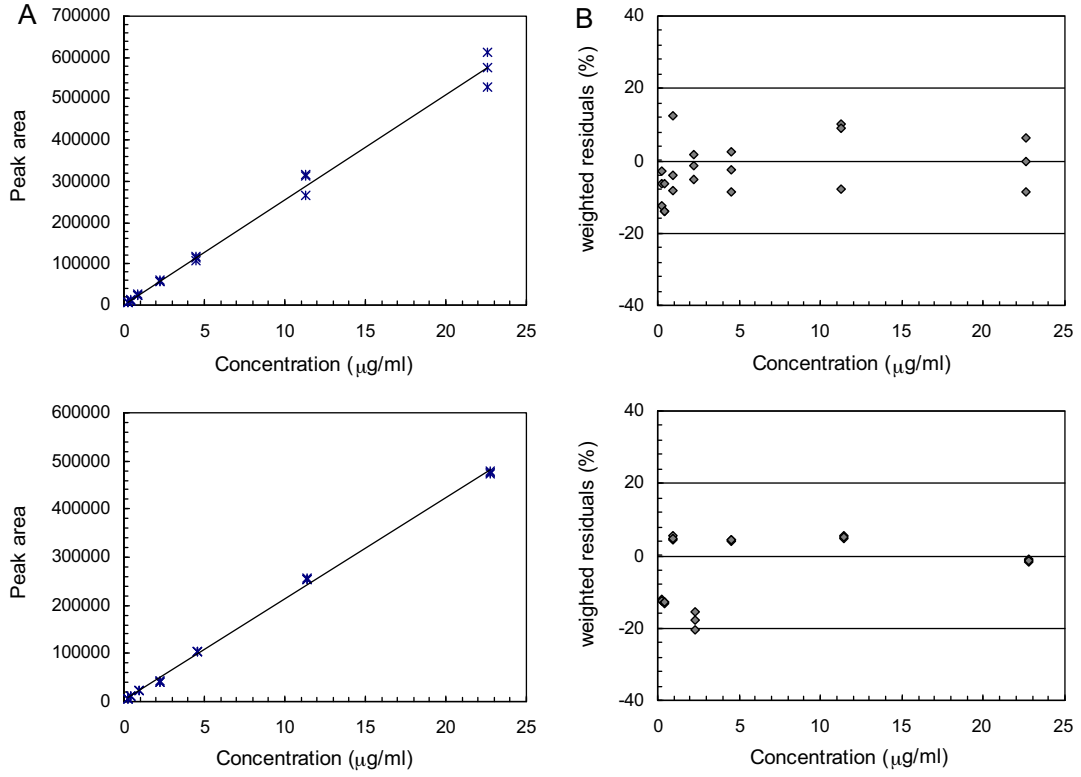

Fig. 2. Calibration curves (A) and weighed residual plots (B) relative to RP-HPLC quantitative analysis of SVL (top) and SVA (down) (5 points, $n = 3$).

for SVA. The limit of quantitation (QOD) (estimated as 10 times the background noise) was 23.5 μg/L for SVL and 11.3 μg/L for SVA. No significant difference in background noise was observed when analyzing the standards in ethanol and those in water or hydroalcholic solutions. Therefore, the QOD limit was assumed to be the same independently of the solvent used.

The accuracy and precision of the proposed method was demonstrated by performing RP-HPLC analysis of standard mixtures of SVL and SVA (3 concentrations; 5 replicates; $n = 15$). The range, relative percent error, standard deviation and relative standard deviation (%) calculated at each concentration level are given in Table 2 (CL 95%). The relative standard deviation values, which are less than 2% for the three level studied, indicated the high reproducibility of the method. The relative percent error always around 5% confirmed the accuracy of the proposed procedure.

To validate daily the performance of the analytical method, SVL and SVA solutions used to perform experiments were analyzed by RP-HPLC. Peak areas were compared with those of pure analytical standards in ethanol. The recoveries were always greater than 99%. Replicate analyses (3 replicates) of standards containing different amounts of SVL and SVA at 4 day intervals gave good reproducibility (CL 95%; RSD < 3%) ensuring the inter-day repeatability of the analytical method. Random analyses of SVL/SVA standards performed by different HPLC equipments and analysts further supported these conclusions (data not shown).

3.2. Complexation of SVL and SVA with HPβCD

UV studies on either SVL or SVA were conducted to measure stability constants of both SVL/HPβCD and SVA/HPβCD complexes as well as to gain indications on their stoichiometry. As can be seen in Fig. 3, a straight line was achieved by plotting [Dr][HPβCD]/ΔAbs *versus* [HPβCD] according to Scott's Eq. (1) for both SVL and SVA

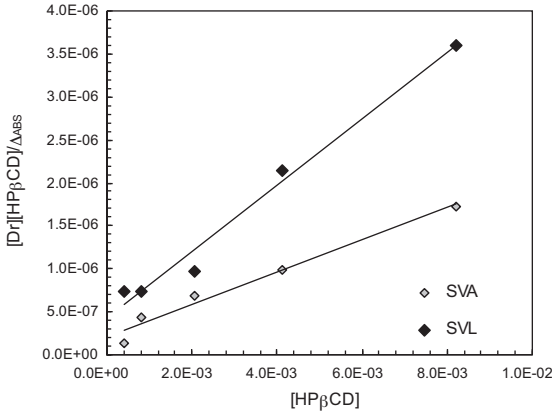

Fig. 3. Variation of SVL and SVA absorbance at 238 nm as a function of HPβCD concentration. Data were fitted according to the Scott's Eq. (1).

($r^2 > 0.98$), suggesting a 1:1 mol/mol complex stoichiometry. Interestingly, no significantly different values of k_f were obtained for SVL ($k_{f1} = 8.90 \times 10^2\ M^{-1}$) and SVA ($k_{f2} = 9.05 \times 10^2\ M^{-1}$) (CL 95%, $p \geq 0.05$).

NMR spectra of SVA, HPβCD and their inclusion complex are shown in Fig. 4. The NMR signals indicated with * were assigned to vynilic protons and to CH(OH) belonging to the unsaturated byciclic ring system of simvastatin. The two peaks between 4.3 and 3.9 ppm were assigned to the CH(OH) moieties of the open lactone ring and labelled with +. The *-marked peaks underwent remarkable downfield shift upon complexation with HPβCD, thus indicating that the fused ring system was significantly interacting with the host cavity. Conversely, the +-marked protons belonging to the open lactone ring – at least the one not overlapped to HPβCD resonances – did not seem to show variations in chemical shift induced by complexation.

Table 2
Accuracy and precision of SVL/SVA quantitative RP-HPLC analysis.

	SVL			SVA		
	Std A	Std B	Std C	Std A	Std B	Std C
Theoretical concentration μ ($\mu g\,mL^{-1}$)	0.242	2.42	24.2	0.262	2.62	26.2
Concentration found ($\mu g\,mL^{-1}$)	0.254	2.31	25.4	0.276	2.48	27.5
|Range| ($\mu g\,mL^{-1}$)	0.01	0.05	0.74	0.01	0.04	0.20
|Error| (%)	4.88	4.73	4.85	5.48	5.22	4.88
SD ($\mu g\,mL^{-1}$)[a]	±0.003	±0.02	±0.5	±0.003	±0.02	±0.5
RSD (%)[a]	±1.33	±1.06	±1.85	±1.00	±0.77	±1.79

[a] Five replicates were analysed at each concentration level ($n = 15$).

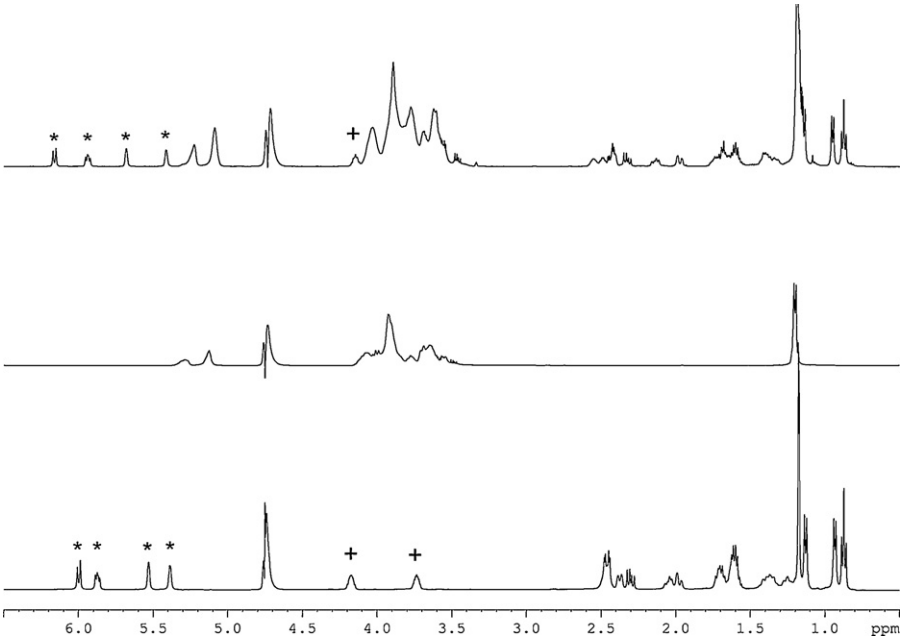

Fig. 4. NMR spectrum in D_2O of SVA (bottom), HPβCD (medium) and SVA/HPβCD 1:1 mol/mol (top).

3.3. Effect of HPβCD on SVL hydrolysis

Hydrolysis of SVL along time in media without or with different molar ratios of HPβCD are reported in Fig. 5. SVL gradually turned into SVA at different rates depending on the presence of HPβCD in the solution. Significantly different data were achieved in water containing HPβCD (CL 95%, $p < 0.001$ for SVL *versus* SVL/HPβCD), which appeared to accelerate SVL hydrolysis. On the basis of previous findings (Alvarez-Lueje et al., 2005; Loftsson and Duchene, 2007), a pseudo-first order kinetic reversible model (Eq. (2)) was assumed to drive simvastatin hydrolysis and used to fit experimental data. In the presence of HPβCD, an approximately 2-fold increase of the hydrolysis constant (k_h for SVL of 0.87 h^{-1} and k_{h_CD} for SVL/HPβCD of 2.08 h^{-1}, respectively) was observed.

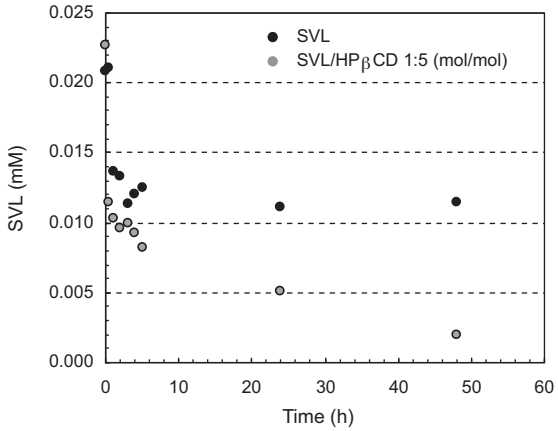

Fig. 5. Hydrolysis kinetics of SVL in water and water containing HPβCD (1:5 mol/mol). Data were fitted according to a first-order kinetic model (Eq. (2)).

3.4. Effect of HPβCD on drug equilibrium solubility and drug dissolution

The solubility profile of SVL after 4 days of incubation with different amounts of HPβCD is reported in Fig. 6. As it can be seen, the amount of dissolved SVL (assessed as SVL *plus* SVA) increased along time due to the presence of HPβCD. Nonetheless, the contemporary measurement of the amount of SVA formed in water during the experiment allowed to assess that percent SVA in solution at equilibrium progressively decreased as HPβCD concentration increased.

The amount of simvastatin and simvastatin/HPβCD powders (1:1 mole ratio) prepared by kneading (KN) and spray drying (SD) dissolved after 30 min at physiological pH are shown in Fig. 7. As it can be seen in Fig. 7, the effect of HPβCD on the total amount of SVL in solution from both KN and SD was moderate for KN and pronounced for SD. This effect, typical of binary systems when a drug/CD interaction in the solid state takes place, was related to the

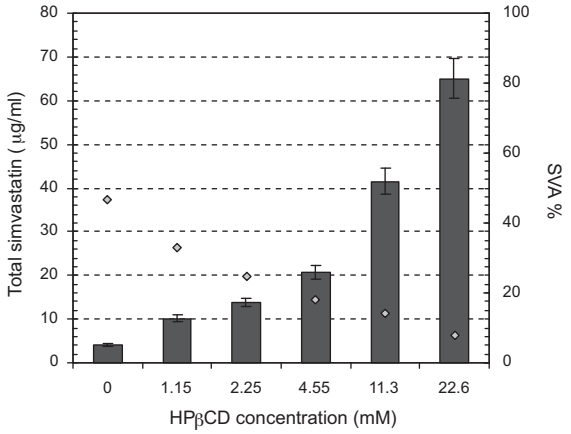

Fig. 6. Simvastatin solubility in the presence of increasing amounts of HPβCD after 4 days. Bars: total simvastatin concentration (SVL *plus* SVA). Diamonds: percent SVA.

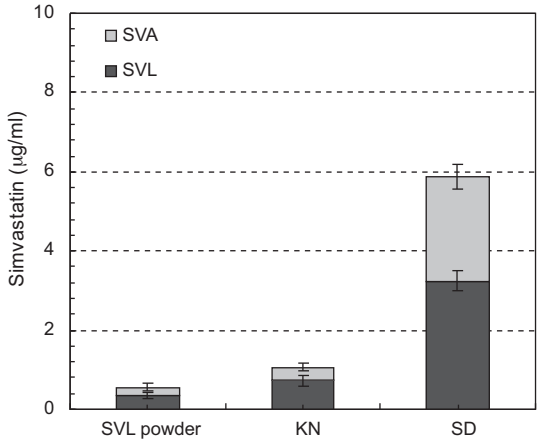

Fig. 7. Dissolution in phosphate buffer at pH 7.4 of simvastatin (A), simvastatin/HPβCD kneaded (B) and spray-dried (C) products after 30 min.

crystallinity of SVL which was in the order SVL > KN > SD as demonstrated by DSC analysis (Fig. 8). In the case of KN, the presence of HPβCD had no effect on the amount of SVA found in solution as compared to simvastatin powder. On the other hand, the amount of SVA found in solution upon SD dissolution was highly increased. In all the cases, conversion of SVL to SVA was found almost constant.

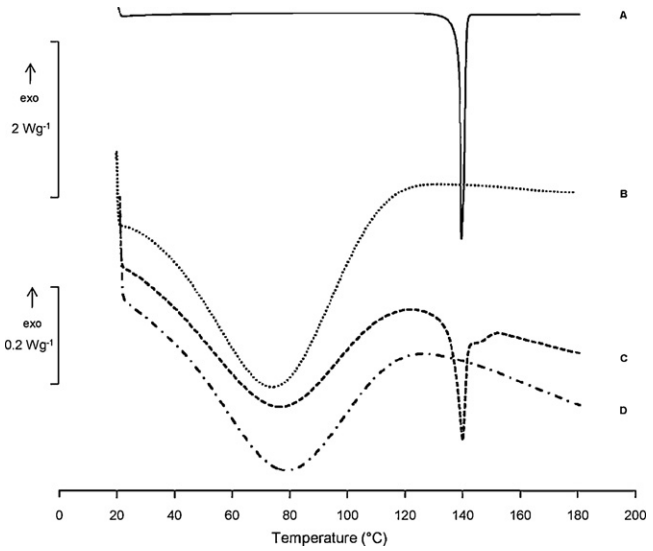

Fig. 8. DSC thermograms of simvastatin (A), HPβCD (B), simvastatin/HPβCD kneaded (C) and spray-dried (D) products.

4. Discussion

Classical preformulation studies on CD-based dosage forms provide for a deep investigation of CD/drug interactions in aqueous solution and in the solid state. When dealing with a labile molecule such as simvastatin, existing in solution as both inactive pro-drug (i.e., SVL) and active form (i.e., SVA), these studies may fail in determining the actual effect of CD as solubility enhancer, due to the presence of multiple aqueous equilibria. To examine closely the effect of HPβCD on SVL/SVA aqueous equilibrium and highlight some potential pharmaceutical implications, the interactions between both forms of simvastatin and the complexing agent occurring in aqueous solution were investigated in depth. First, we developed a simple and convenient method for simultaneous determination of SVL and SVA. Then, we tried to understand if HPβCD affected SVL hydrolysis, equilibrium solubility and dissolution, which are aspects undoubtedly related to availability at absorption/action site.

To date, the analytical technique of choice for the quantitation of SVL and SVA in biological samples is HPLC in combination with tandem mass spectrometry (MS/MS) (Yang et al., 2003; Barrett et al., 2006; Apostolou et al., 2008). Actually, due to their sensitivity and specificity, HPLC-MS methods well fit simvastatin determination in sparely clean multicomponent matrices, such as human plasma (Jemal and Ouyang, 2000; Nirogi et al., 2007). Nevertheless, HPLC/UV methods can be more friendly employed for a rapid

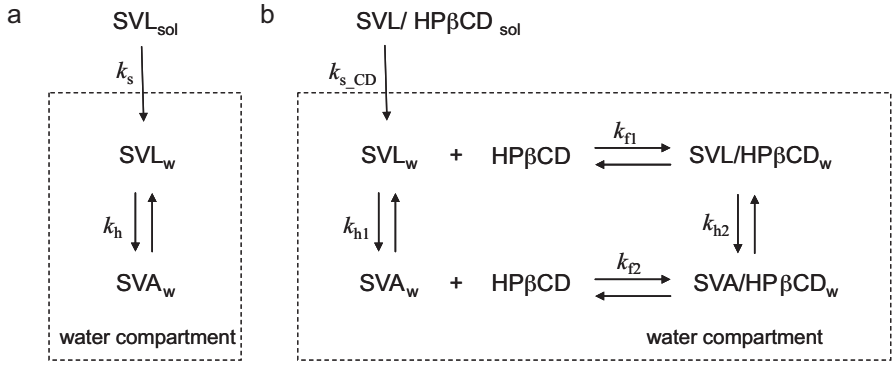

Fig. 9. Schematic representation of SVL/SVA equilibria occurring in water in the absence (A) and in the presence (B) of HPβCD.

and simple laboratory analysis of simvastatin formulations. Ph.Eur. VI Ed. (EDQM, 2007) reports a chromatographic procedure to test simvastatin purity in term of percentage of lovastatin and epilovastatin present in the sample. Notably, the analytical method does not take into account SVL/SVA equilibrium in aqueous solution. To address this issue, a sensitive, precise and accurate RP-HPLC method with UV detection able to discriminate between SVL and SVA was developed, validated and applied to study the effect of HPβCD on SVL/SVA equilibrium.

Stability of SVL alone or in the presence of HPβCD in water at pH 5.5 highlighted that SVL gradually turned into SVA at a higher rate in the presence of HPβCD, despite complex formation. In fact, stability constant of SVL and SVA with HPβCD, as evaluated by UV spectrophotometry after assessing the identity of the guests by RP-HPLC, gave comparable values for SVA and SVL, suggesting that both the lactone and the open-ring form of simvastatin interact with HPβCD in a similar extent. Furthermore, NMR results indicated that the lactone ring was not, or only partly, included in the HPβCD hydrophobic cavity and therefore the lactone ring was not directly involved in the formation of the inclusion complex. This hypothesis well agree with that of Szente and colleagues [19], who suggested an interaction of the 2,2-dimethyl butanoic acid ester group on C8' of simvastatin in CD hydrophobic cavity.

Measurement of drug apparent solubility at different CD concentrations is the traditional and probably reference method to assess the stability constant of drug/CD complexes (Higuchi and Connors, 1965; Loftsson and Brewster, 1996; Brewster and Loftsson, 2007). The phase–solubility profile is constructed by assessing CD effect on the total drug concentration in solution at equilibrium as determined by appropriate analytical techniques, typically UV–vis spectrophotometry. To date, also simvastatin/CD complexes equilibrium solubility has been assessed through simvastatin quantitation by UV spectrophotometry (Jun et al., 2007; Patel and Patel, 2007; Yoshinari et al., 2007; Sule et al., 2009), thus neglecting the transformation of dissolved SVL in SVA. In this work, HPLC analysis was used to distinguish the amounts of SVL and SVA in CD-containing solutions. Notably, different values of stability constant could be calculated from Eq. (3) for SVL/HPβCD complex when considering either actual SVL ($1.14 \times 10^3\ M^{-1}$) or total simvastatin (i.e., SVL *plus* SVA) dissolved ($5.71 \times 10^2\ M^{-1}$). As expected, this last value is more consistent with literature reports (Jun et al., 2007; Patel and Patel, 2007; Sule et al., 2009) but far from the values calculated from the UV method reported above. Thus, results of HPLC-assisted solubility studies further highlight that particular attention must be paid when evaluating the extent of interaction between CDs and unstable drugs by classical UV-assisted methods.

Along with equilibrium solubility, drug dissolution is a parameter of utmost importance when considering bioavailability of poorly water-soluble actives such as SVL. Again, if one considers the total amount of simvastatin dissolved, HPβCD demonstrates its expected hydrotropic activity. However, taking into account the contemporary transformation of dissolved SVL in SVA, it could be evidenced that CD solubilizing effect is accompanied by the formation of SVA.

On the basis of the experimental data collected, a hypothesis on the effect exerted by HPβCD upon dissolution of simvastatin powders and, consequently, upon SVL/SVA chemical equilibrium in solution was formulated. As reported in Fig. 9A, when SVL powder (SVL_{sol}) is placed in water, two chemical processes are instantaneously established, that is SVL dissolution (leading to SVL_w) and its progressive hydrolysis to SVA_w. The resultant amounts of SVL and SVA in water will depend on both powder dissolution properties (and thus solubility at equilibrium and dissolution constant k_s) and SVL hydrolysis constant k_h. For a slightly soluble molecule, such as simvastatin, the rate-limiting step for SVA_w appearance in water would be SVL_s dissolution. In the case of a SVL/HPβCD powder, assuming a faster HPβCD dissolution, both dissolved SVL (SVL_w) and SVA (SVA_w) may interact with HPβCD forming SVL/HPβCD and SVA/HPβCD complexes with comparable stability constants (i.e., k_{f1} and k_{f2}, respectively) (Fig. 9B). SVL_w and SVA_w amounts are expected to be dependent on both powder solubility at equilibrium, dissolution constant and SVL hydrolysis constant as affected by HPβCD. As demonstrated above, all these values are increased by HPβCD addition. The shift of SVL_w/SVA_w equilibrium toward SVA_w in the presence of HPβCD can be interpreted considering that hydrolysis occurs not only on free SVL_w (k_{h1}) but also on $SVL/HP\beta CD_w$ complex at a rate k_{h2}, giving an overall k_{h_CD} higher than k_h. This can be well hypothesized assuming a simvastatin/CD mode of interaction which does not involve the lactone ring, as suggested by NMR studies and previous literature findings (Sule et al., 2009). Thus, the improved SVL dissolution profile may be likely ascribed to SVL conversion in the more soluble SVA as well as the occurrence of SVL/HPβCD complex in water. This view applies also when describing SVA/SVL equilibria occurring in HPβCD-containing solutions, i.e., when dissolution of solid drug is not involved.

From a pharmaceutical point of view, experimental data suggest that the contribution of HPβCD in increasing simvastatin solubility is predominant on its ability to promote SVA formation. Meanwhile, a conversion of SVL to SVA in a constant extent during simvastatin dissolution gives the highest amount of dissolved SVA in the case of the more soluble SVL/HPβCD complex. Thus, it can be hypothesized that this behaviour well occur also *in vivo* after oral administration. HPβCD should increase the amount of SVL dissolved and in so doing be of great benefit to achieve a faster simvastatin adsorption and likely to avoid massive first-pass effect (i.e., increased bioavailability). Nonetheless, the contemporary capability of HPβCD to promote SVA transformation may induce a premature conversion of SVL to SVA, which is undesirable due to SVA lower absorption extent and liver selectivity. On the other hand, conceiving CD-based drug delivery systems for local simvastatin applications (e.g., bone formation), the conversion of SVL in SVA induced by HPβCD may be desirable to elicit an *in situ* long-term exposure to the active hydroxyacid form.

5. Conclusions

An UV-assisted RP-HPLC method able to selectively identify and quantify SVL and SVA in solution has been set up and successfully applied to follow SVL hydrolysis into SVA in the presence or not of the hydrosolubilizing excipient HPβCD. Results have shown that HPβCD, generally employed as stabilizing agent, surprisingly promotes SVL conversion to the hydroxyacid form in aqueous solution. The results also suggest that the lactone and the open-ring form of simvastatin interact with HPβCD in a similar extent and that hydrolysis occurs also on SVL/HPβCD complex. Finally, particular attention must be paid to SVL lactonization in aqueous solution when using CD-based formulations and in demonstrating their effective benefit for a specific therapeutic use.

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