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ORIGINAL ARTICLE

Synthesis and characterization of the inclusion complex between repaglinide and sulfobutylether-β-cyclodextrin (Captisol®)

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Abstract An inclusion complex between repaglinide and a cyclodextrin derivative, 7-sulfobutylether-β-cyclodextrin (Captisol®) was synthesized and characterized taking into account its possible benefits regarding the solubility, thus the bioavailability of repaglinide (an oral antidiabetic, carbamoyl methyl benzoic acid, practically insoluble in water, used for the treatment of type II diabetes mellitus). In order to establish the optimal conditions for the synthesis, phase-solubility studies were performed. Their results (A_L type phase-solubility diagram), as well as the chromatographic retention behavior of repaglinide in the presence of Captisol® indicated that an 1:1 inclusion complex is formed. The estimated apparent stability constant, according to the Higuchi-Connors method is 530 M^{-1}. These data were confirmed by ^{1}H-NMR, IR, DTA of the inclusion complex prepared through lyophilization.

Keywords Repaglinide · Inclusion complex · Sulfobutylether-β-cyclodextrin · Phase-solubility

Introduction

Cyclodextrins (cyclic oligosaccharides consisting of six (α-cyclodextrin), seven (β-cyclodextrin), eight (γ-cyclodextrin) or more glucopyranose units linked by α-(1,4) bonds, also known as cycloamyloses, cyclomaltoses and Schardinger dextrins [1]) serve as multi-functional pharmaceutical excipients due to their special ability to complex with drugs and modify their physical, chemical and biological properties [2, 3]: increase solubility [4–6], improve taste and odor, enhance stability [7–9] and decrease tissue irritation upon dosing, but mostly, enhance bioavailability of the guest molecules [10, 11].

Apart from the naturally occurring cyclodextrins, many cyclodextrin derivatives have been synthesized, with variable solubility, depending on the substituent.

Repaglinide, 2-ethoxy-4-[2-[[(1S)-3-methyl-1-[2-(1-piperidinyl)phenyl]butyl]amino]-2-oxoethyl]benzoic acid, is a short-acting insulin oral secretagogue. Repaglinide lowers the blood glucose levels acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning β-cells in the pancreatic islets.

Repaglinide (Fig. 1) closes ATP-dependent potassium channels in the β-cell membrane via a target protein different from other secretagogues. This depolarises the β-cell and leads to an opening of the calcium channels. The resulting increased calcium influx induces insulin secretion from the β-cell [12]. Repaglinide, an acid–base ampholyte ($pK_{a1} = 4.2$; $pK_{a2} = 6.0$) exhibits very low water solubility (34 μg mL^{-1} at 37 °C) and high lipophilicity (log P = 3.97) [13], which probably are responsible for the major side-effects of repaglinide, as for all meglinides, both hyperglycemia and hypoglycemia, and a relatively variable bioavailability [12].

Sulfobutylether-β-cyclodextrin (Fig. 2) is a polyanionic variably substituted cyclodextrin derivative ($(SBE)_{7m}$-β-CD, Captisol®), with an average degree of substitution 6.5; the renal toxicity of parent cyclodextrins was dramatically reduced by substitution. The aqueous solubility of the neutral, cationic and anionic drugs complexed by $(SBE)_{7m}$-β-CD increased by a factor of minimum 10 [14].

As published elsewhere [15–17], the interaction between repaglinide and β-cyclodextrin and some of its derivatives (randomly methylated β-cyclodextrin (RAMEB) and

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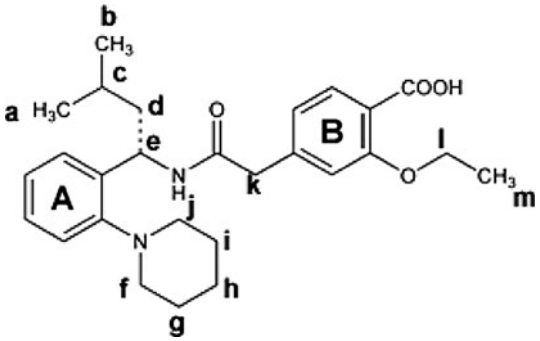

Fig. 1 Repaglinide

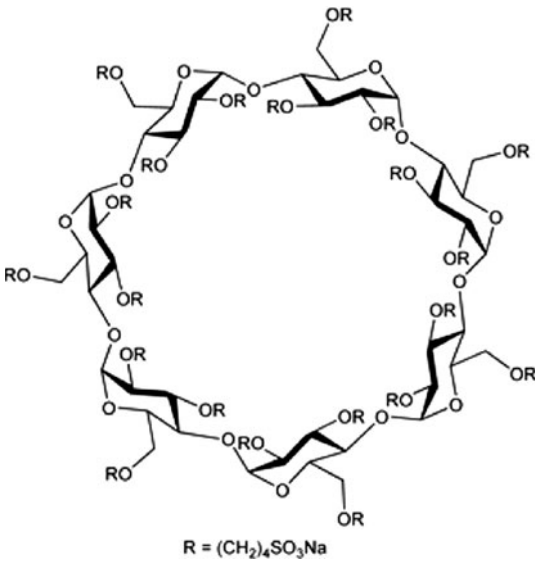

Fig. 2 Captisol® $(SBE)_{7m}$-β-CD

hydroxypropyl-β-cyclodextrin (HP-β-CD)) was already studied and evidence that 1:1 inclusion complexes are formed was obtained. After applying several preparation methods (lyophylisation, co-precipitation and kneading method), the most efficient method proved to be the lyophylisation. The formed inclusion complexes have been characterized by means of Nuclear Magnetic Resonance (^{1}H-NMR), Differential Scanning Calorimetry (DSC), Infrared Spectroscopy (FT-IR) and phase-solubility diagrams were determined.

β-CD and its derivatives enhance the solubility of repaglinide: HP-β-CD gives a soluble complex, while in the presence of β-CD and RAMEB, inclusion complexes with limited solubility are formed. The calculated apparent stability constants indicate the decrease of the repaglinide inclusion complexes stability as follows: HP-β-CD:repaglinide > RAMEB:repaglinide > β-CD:repaglinide [17].

The aim of the present study is to investigate the possibility of complexation of repaglinide with $(SBE)_{7m}$-β-CD. The complex was prepared by using different methods at stoichiometric ratios. Selective physicochemical determinations based on differential thermal analysis (DTA), NMR and FT-IR were used to characterize the inclusion complex. Phase-solubility studies were performed according to the method reported by Higuchi and Connors [18]. We also examined the chromatographic behavior of repaglinide in a reversed-phase system involving the formation of $(SBE)_{7m}$-β-CD inclusion complex in the mobile phase. The effect of the concentration of $(SBE)_{7m}$-β-CD in the mobile phase on the retention factor will be discussed. An attempt is made to estimate the stoichiometry and the apparent formation constant of $(SBE)_{7m}$-β-CD inclusion complex from the relationship between the retention factor and the $(SBE)_{7m}$-β-CD concentration in the mobile phase [19, 20].

Materials and methods

Reagents and apparatus

Repaglinide, with purity of 97.69% without crystal water was obtained from Novo Nordisk A/S, Denmark and Sulfobutylether-β-cyclodextrin (average substitution degree 6.5), from CyDex Pharma (USA). Potassium dihydrogenphosphate, phosphoric acid, sodium hydroxide, potassium nitrate were of analytical grade (Merck KgA, Germany). HPLC grade acetonitrile was purchased from Merck KgA, Germany. Deionized ultrapure water for chromatography was used as a solvent (prepared using a Barnstead Easy-Pure RoDi apparatus). The solvent DMSO d_6 was of spectroscopic NMR grade. All the other reagents used were of analytical grade.

10 mM phosphate buffer solutions were prepared using H_3PO_4 85% solution and NaOH 1 M solution. The pH values (3.00; 4.00; 5.00; 6.00; 7.00 and 8.00) were adjusted under potentiometrical control, using a Metrohm 716 DMS Titrino potentiometer. For the solubility studies we used a Vortex-Genie®2 (Scientific Industries) shaker and a Perkin-Elmer Lambda 2 UV/VIS Spectrometer. IR spectra were recorded on a FT-IR Bio-Rad FTS155 instrument, using the KBr sample preparation method. The lyophilization was performed in an Alpha 1-2/LD2-2, Martin Christ liophyliser.

^{1}H-NMR and ^{13}C-NMR spectra were recorded at 25 °C on a 300 MHz GEMINI-300BB instrument, using DMSO-d_6 for sample solubilization. The spectra recordings were characterized by a digital resolution of 0.56 Hz/point, 16 K datapoints, a sweep width of 4500 Hz and an acquisition time of 1.998 s. All chemical shifts were measured relative to TMS.

Thermal analysis was carried out using a TGA/SDTA 851-LF 1100 Mettler apparatus. The thermal behaviour of samples was studied under a nitrogen flow of 50 mL min^{-1}

in the temperature range of 25–1000 °C for the heating rate of 10 °C min^{-1}. Samples with mass of about 10 mg were packed in Pt crucibles of 150 μL.

Methods

Preparation of the inclusion complexes

0.375 mmol sulfobutylether-β-cyclodextrin were dissolved in 50 mL distilled water. An ethanolic solution containing 0.375 mmol repaglinide was added stepwise to the aqueous solution of sulfobutylether-β-cyclodextrin. The suspension was stirred mechanically for 24 h, cooled for approx. 48 h at 2–8 °C and another 24 h at −20 °C and finally lyophilized at −60 °C for 24 h.

Phase-solubility analysis

Phase-solubility study was carried out according to the method of Higuchi and Connors [19]. Excess amount of repaglinide (approx. 10 mg) was added to 2.5 mL 10 mM phosphate buffer (pH 6 and pH 3.5) containing increasing amounts of Captisol® (10^{-3}–10^{-1}M) and shaken at 25 ± 0.5 °C.

In order to establish the optimal mixing time, vials were shaken up to 72 h, and sample absorbance was measured every 12 h. The comparative results showed no significant differences for shaking times exceeding 24 h. Hence, the shaking time was set to 24 h; further, the suspensions were equilibrated 24 h. Samples were filtered through a 0.2 μm Nylon filter membrane (Whatman® Puradisc™), the absorbance at λ 283 nm (Fig. 10) was measured and the concentration of the dissolved repaglinide was determined. The experiments were conducted in triplicate.

The apparent stability constant of the complex was calculated from the phase-solubility diagram, according to the following equation:

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

where S_0 is the solubility of repaglinide at 25 °C in the absence of cyclodextrin, fairly approximated from the y-intercept.

HPLC

Experiments were carried out using a Varian high performance liquid chromatograph with a Prostar 240 quaternary pump with a Rheodyne 7725i manual injection valve with a 20 μL sample loop, and a photodiode array detector (Prostar 330). For data acquisition we used a Star Chromatography WorkStation 6.6 software. A C18 silica Inertsil 5 ODS-2 column (250 × 4.6 mm i.d., 5 mm particle size) provided by Varian ChromSep was used. Isocratic mobile phase elution was performed using a 50/50 v/v mixture of pH 3.5 aqueous phosphate buffer (10 mM) and acetonitrile containing $(SBE)_{7m}$-β-CD in the concentration range 0.000–0.020 M, with a 1 mL min^{-1} flow rate. The detection wavelength was 240 nm with full spectra recorded between 220 and 400 nm; data acquisition channels were 254, 240 and 283 nm (240 and 283 nm are the specific wavelengths for repaglinide absorption maxima in the UV absorption spectrum).

Results and discussion

Characterization of the inclusion complexes

^{1}H-NMR spectra

In the ^{1}H-NMR spectra of the formed inclusion compounds, repaglinide protons showed an upfield displacement and a downfield displacement (for the ring A) due to a variation of local polarity and, also to the weak interactions with CD cavity hydrogen atoms. Figures 3 and 4 show the ^{1}H-NMR spectra obtained for repaglinide and for its inclusion complex with $(SBE)_{7m}$-β-CD.

In Table 1, ^{1}H NMR assignments, multiplicity, the chemical shifts and their changes upon complexation for the repaglinide protons are presented, where $\Delta\delta = \delta$ complex – δ free repaglinide

The NMR spectroscopic analysis suggested that the aromatic ring A from the repaglinide structure (a highly hydrophobic part of the molecule) displays the most favourable interaction with the hydrophobic cavity of $(SBE)_{7m}$-β-CD, based on the highest values of changes in the chemical shifts of the protons of this nucleus: $\Delta\delta$: 0.47–0.24 ppm. The steric factor plays also an important role in the inclusion process. According to results presented elsewhere [8], higher variations of chemical shifts have been registered for the unsubstituted molecule of β-CD, while other β-CD derivatives, with the larger substituents, like HP-β-CD for example, show lower changes in chemical shifts for the same protons. The same behavior was noticed for the $(SBE)_{7m}$-β-CD–repaglinide complex. This proves that the inclusion into the cyclodextrin cavity of the hydrophobic parts of repaglinide can be hindered by the presence of large substituents placed on the glucose units of the β-CD molecule.

IR spectra

The IR spectrum of repaglinide reveals the presence of a peak at 3293.12 cm^{-1}, assigned to N–H stretching vibration and one at 1672.44 cm^{-1}, corresponding to the carbonyl group.

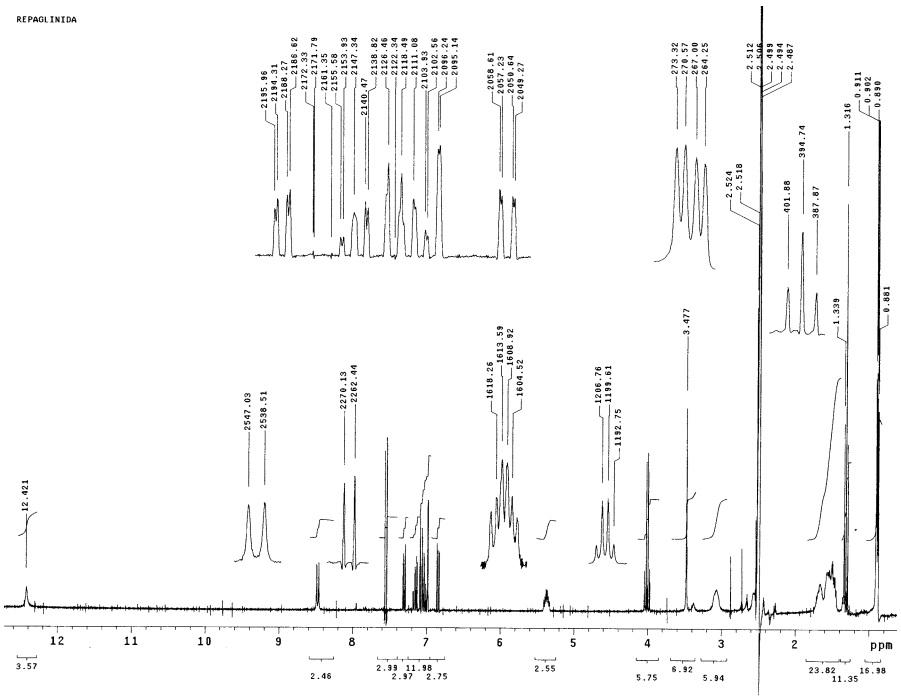

Fig. 3 ^{1}H-NMR spectrum of repaglinide

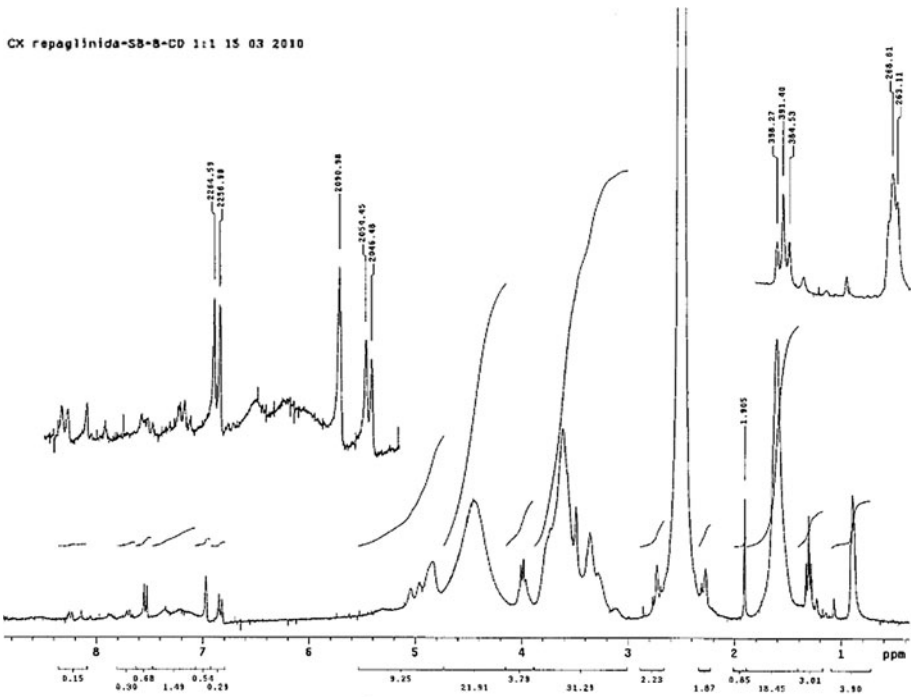

Fig. 4 ^{1}H-NMR spectrum of $(SBE)_{7m}$-β-CD–repaglinide inclusion complex

The spectrum of $(SBE)_{7m}$-β-CD is characterized by intense bands at 3300–3500 cm^{-1} due to O–H stretching vibration, while the vibration of the –CH and $–CH_2–$ groups appears in the 2800–3000 cm^{-1} region.

Upon complexation, the repaglinide absorption peak at 3293.12 cm^{-1} was not identified any more in the IR spectra due to the appearance of host–guest interactions. These suggest the possibility of formation of hydrogen bonds between the hydroxyl groups of the host cavity and the repaglinide carbonyl group. In Fig. 5 the IR spectra for: repaglinide and $(SBE)_{7m}$-β-CD–repaglinide inclusion complex are presented.

Thermal analysis

The thermal analysis of repaglinide revealed a single, sharp endothermic peak at 134.04 °C (melting point) (Fig. 6), while the DTA curve for $(SBE)_{7m}$-β-CD is characterized

Table 1 ^{1}H NMR assignments, multiplicity, chemical shifts, δ (ppm) and chemical shifts variations for repaglinide protons

Repaglinide δ (ppm, multiplicity)	$(SBE)_{7m}$-β-CD–repaglinide δ (ppm, multiplicity)	$\Delta\delta$ (ppm)
0.89 (d) (*a*,*b*)	0.88 (d) (*a*,*b*)	−0.01
1.32 (t) (*m*)	1.31 (t) (*m*)	−0.01
1.40–1.85 (m) (*g*,*h*,*i*)	Not identified	–
4.01 (q) (*l*)	4.01 (q) (*l*)	0.00
6.85–6.99 (dd, d) (*ring B*)	6.82–6.98 (*ring B*)	−0.03 to −0.01
7.29–7.32 (td, dd) (*ring A*)	7.52–7.55 (*ring A*)	0.23

d dublet, *dd* double dublet, *m* multiplet, *q* quartet, *t* triplet, *td* triple dublet

by broad endothermic effect, which attained a maximum up to 100 °C. A possible evidence for the formation of inclusion complex between repaglinide and Captisol® was the change in the shape of the melting peak of repaglinide and its shift in the DTA curve: the peak corresponding to the melting of the drug substance was observed around 130.9 °C (Fig. 8), as a result of the inclusion of the drug into the cyclodextrin cavity.

Figures 6, 7 and 8 show the DTA, TG and DTG curves for repaglinide, $(SBE)_{7m}$-β-CD and $(SBE)_{7m}$-β-CD–repaglinide inclusion complex.

Phase-solubility analysis

Repaglinide is an acid–base ampholyte, with two protonation sites ($pKa_1 = 4.2$; $pKa_2 = 6.0$). At the isoelectric pH (5.50), two neutral forms of repaglinide (zwitterionic and uncharged form) exist, resulting in a significant lipophilicity of this molecule [13].

In order to establish the optimal pH value of the solution used for the solubility studies, we have investigated the pH influence on the repaglinide solubility in the presence and in absence of $(SBE)_{7m}$-β-CD.

As shown in Fig. 9, the repaglinide solubility is minimum in the 4–6 pH range, corresponding also to a higher lipophilicity. In this pH range we have noticed a more significant influence of Captisol® on repaglinide solubility. Since the measured pH of the mutual mixture of repaglinide and $(SBE)_{7m}$-β-CD in aqueous medium is 5.9–6.2, we performed our tests according to the method reported by Higuchi and Connors [18] using pH 6 phosphate buffer solutions; in this medium the $(SBE)_{7m}$-β-CD effect on solubility is predominant as the complexation is highly favored by the hydrophobicity of the repaglinide.

We have also studied the effect of $(SBE)_{7m}$-β-CD on repaglinide solubility at pH 3.5 (aqueous phosphate buffer solution), in order to give the results obtained in the HPLC studies a term to compare with.

The repaglinide concentration was calculated using the previously determined specific absorbance ($A^{1\%}_{1cm} = 58$ at pH 6 and $A^{1\%}_{1cm} = 60.93$ at pH 3.5). Captisol® does not interfere the measurement, as it can be seen in Fig. 10.

$(SBE)_{7m}$-β-CD enhances repaglinide solubility in water, as it can be noticed from the phase solubility diagrams (Figs. 11, 12).

The phase solubility diagram for repaglinide–$(SBE)_{7m}$-β-CD at pH 6 within the tested concentration range of cyclodextrin displays a linear increase of solubility with the increasing concentration of $(SBE)_{7m}$-β-CD (A_L type diagram, $r = 0.997$), which indicates the formation of a soluble 1:1 inclusion complex.

The binding constant, 532 M^{-1}, was calculated from the slope of the linear phase-solubility plot.

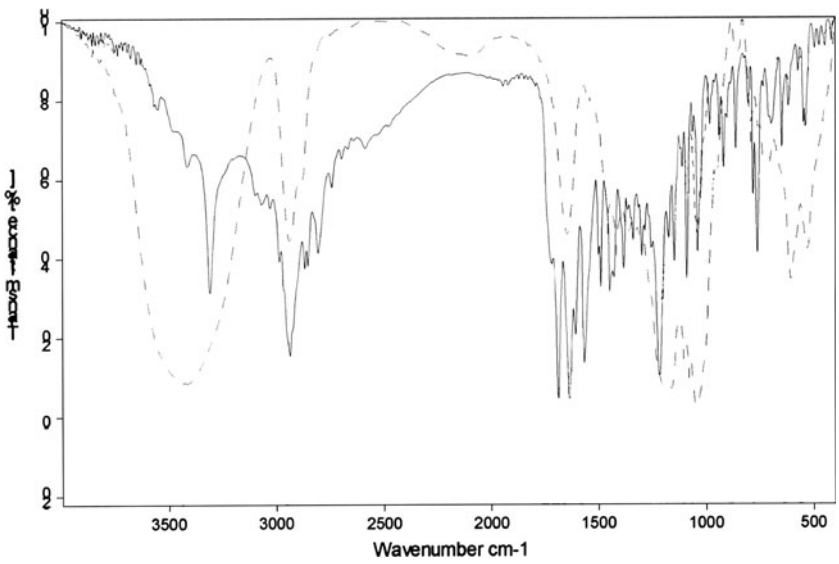

Fig. 5 IR spectra of repaglinide (*solid line*) and repaglinide–$(SBE)_{7m}$-β-CD inclusion complex (*broken line*)

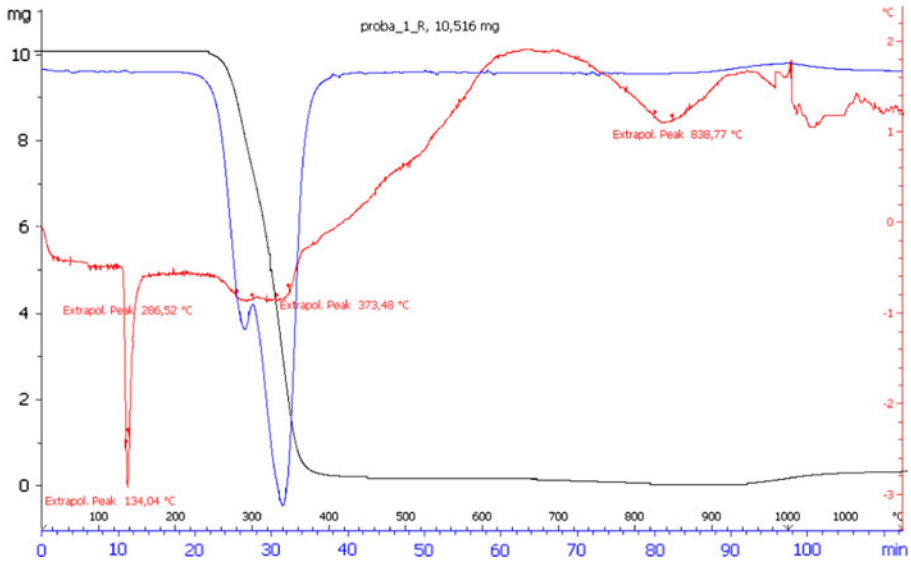

Fig. 6 DTA, TG and DTG curves for repaglinide

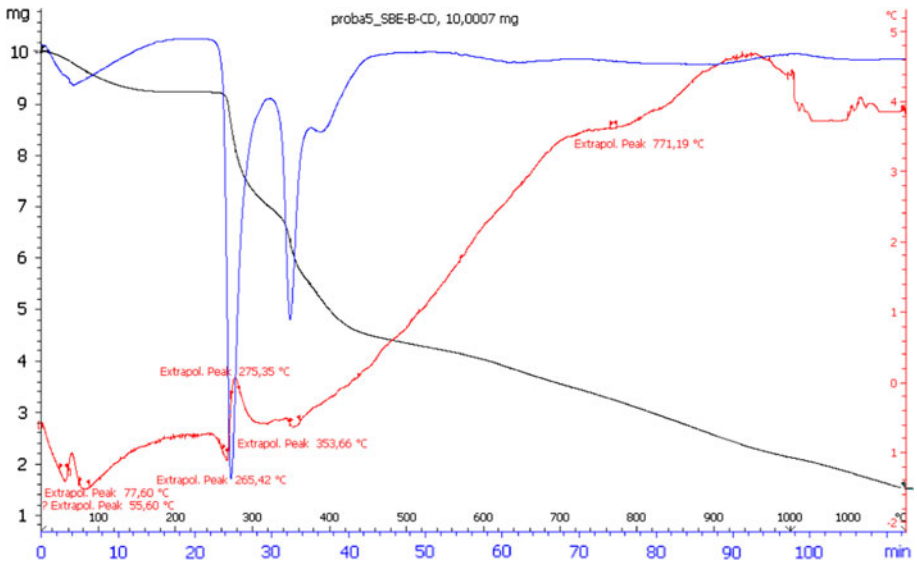

Fig. 7 DTA, TG and DTG curves for $(SBE)_{7m}$-β-CD

HPLC studies

When $(SBE)_{7m}$-β-CD is added to the mobile phase, repaglinide retention is driven by the drug partition between the mobile and stationary phases and the solute complexation with $(SBE)_{7m}$-β-CD. According to the solute retention time and the void time, retention factors were calculated in the presence of increasing concentration of the cyclodextrin. As expected, the retention time decreases as the concentration of the cyclodextrin in the mobile phase increases, because of the reducing residence time in the column of the more hydrophilic inclusion complex of repaglinide. Acetonitrile/phosphate buffer (pH 3.5) was selected as mobile phase because of the good elution ($k' < 10$) of repaglinide. $(SBE)_{7m}$-β-CD effect on repaglinide retention was studied in the concentration range 0.000–0.020 mM and the results are presented in Table 2 and Fig. 13.

When $1/k'$ is plotted versus molar concentration of cyclodextrin in the mobile phase, a linear relationship was observed, indicating a 1:1 stoichiometry of the solute: cyclodextrin complex, and the (slope/intercept) ratio gives the value of K_f 52.5 (Fig. 14). These values are consistent with the decrease in stability noticed in phase-solubility studies carried out in buffered solutions with a pH value of 3.5 (K_f 99, Fig. 12). This even lower stability of the complex is the result of a competition between repaglinide and acetonitrile for the hydrophobic cavity of cyclodextrin.

Conclusions

On the basis of the physicochemical characterization techniques described in this work (NMR, DTA, IR), the

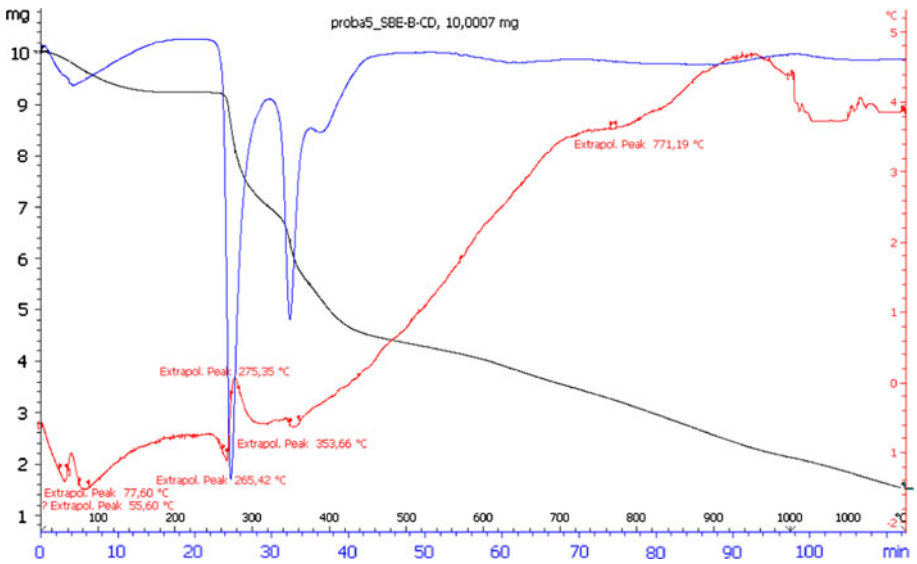

Fig. 8 DTA, TG and DTG curves for the inclusion complex $(SBE)_{7m}$-β-CD–repaglinide

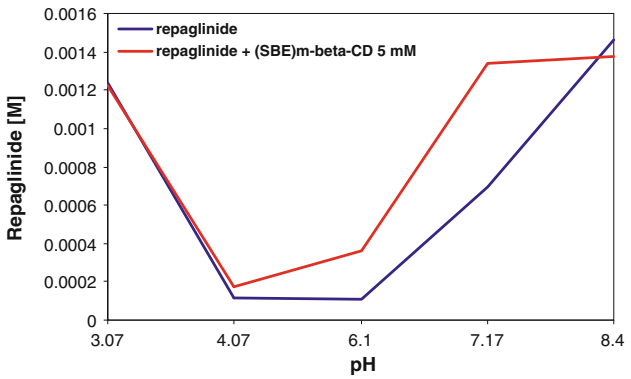

Fig. 9 The pH and Captisol® influence on the repaglinide solubility in water

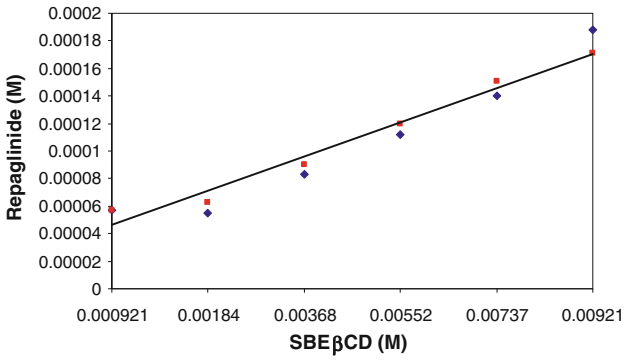

Fig. 11 Phase-solubility diagram of repaglinide–$(SBE)_{7m}$-β-CD system in pH 6 aqueous phosphate buffer at 25 °C

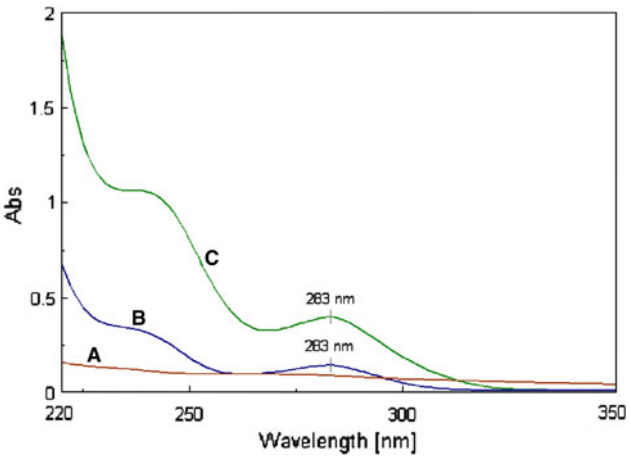

Fig. 10 UV spectra for aqueous solution of repaglinide (B) (2 μg mL^{-1}), $(SBE)_{7m}$-β-CD (A) (20 mg mL^{-1}) and the inclusion complex of repaglinide with $(SBE)_{7m}$-β-CD (C) (sol. sat.)

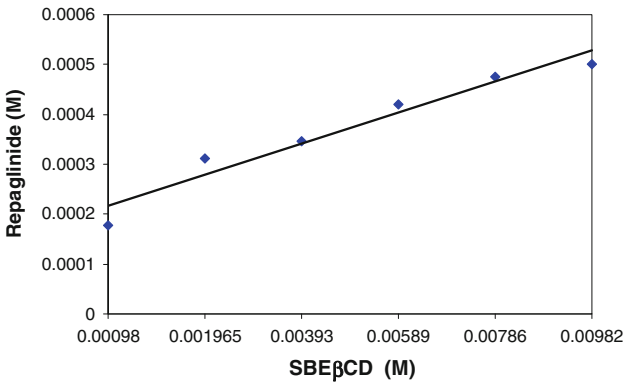

Fig. 12 Phase-solubility diagram of repaglinide–$(SBE)_{7m}$-β-CD system in pH 3.5 aqueous phosphate buffer at 25 °C

complex formation between repaglinide and $(SBE)_{7m}$-β-CD was confirmed.

The aqueous solubility of repaglinide has been improved in aqueous solution of pH 6 through complexation with

Table 2 Retention time and retention factor of repaglinide as function of $(SBE)_{7m}$-β-CD concentration

$(SBE)_{7m}$-β-CD (M)	t_R (min)	k′
0.000	10.06	2.49
0.005	9.09	2.20
0.010	7.88	1.76
0.015	7.30	1.53
0.020	6.78	1.30

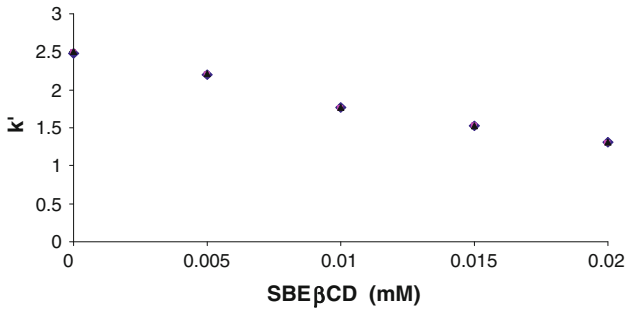

Fig. 13 Effect of cyclodextrin concentration on retention factor of repaglinide

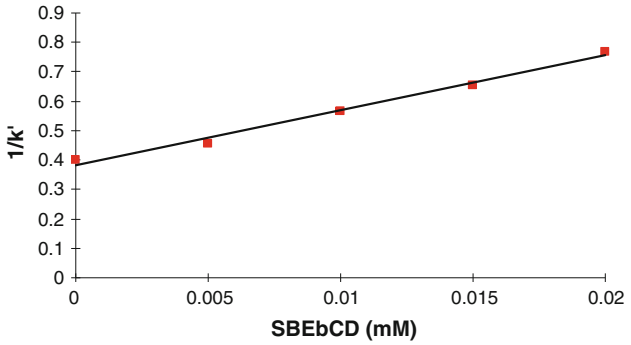

Fig. 14 Reciprocal plot 1/k′ vs. $[(SBE)_{7m}$-β-CD]

Captisol®; the results from the phase-solubility diagram indicate the formation of a 1:1 inclusion complex repaglinide–$(SBE)_{7m}$-β-CD and are consistent with the results in the RP-HPLC study.

Both the stoichiometry and equilibrium constant were evaluated by the phase-solubility method and confirmed by the chromatographic data. Kst. determined from the phase-solubility diagram at a pH value of 6 was 532 M^{-1}, while at pH = 3.5 a decrease in stability of the inclusion complex was observed (Kst. = 99 M^{-1}).

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