PREPARATION AND CHARACTERIZATION OF INCLUSION COMPLEXES BETWEEN REPAGLINIDE AND β – CYCLODEXTRIN, 2 – HYDROXYPROPYL - β – CYCLODEXTRIN AND RANDOMLY METHYLATED β – CYCLODEXTRIN

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Abstract

Repaglinide, an oral antidiabetic drug with short duration of action, administered in patients with type 2 diabetes mellitus exhibits very low water solubility and high lipophilicity. In order to evaluate the possibility of enhancing the drug's solubility, inclusion complexes between repaglinide and β- cyclodextrin(β CD), 2- hydroxypropyl-β cyclodextrin (HP- β-CD) and randomly methylated-β cyclodextrin (RAMEB)) have been obtained by applying the following preparation methods: liophylisation, co-precipitation followed by filtration of the end-product and kneading method.

The inclusion complexes were characterized by means of Nuclear Magnetic Resonance (^{1}H-NMR, ^{13}C-NMR), Differential Scanning Calorimetry (DSC) and Infrared Spectroscopy (FT-IR). The results obtained confirmed the inclusion of repaglinide into the cyclodextrins cavity.

Rezumat

Repaglinida, un antidiabetic oral cu durată scurtă de acţiune utilizat în tratamentul pacienţilor cu diabet zaharat de tip 2, este caracterizată prin solubilitate scăzută în apă şi lipofilie accentuată. Pentru evaluarea posibilităţii de mărire a solubilităţii acestui medicament în apă au fost obţinuţi complecşi de incluziune ai repaglinidei cu β-ciclodextrina (β CD), 2- hidroxipropil-β ciclodextrina (HP- β-CD) şi β ciclodextrina cu procent variabil de metilare (RAMEB). Metodele de obţinere utlizate au fost: liofilizarea, coprecipitarea urmată de filtrare şi metoda triturării.

Complecşii de incluziune formaţi au fost caracterizaţi prin metode spectroscopice (^{1}H-NMR,^{13}C-NMR, FT-IR) şi prin analiză termică: calorimetrie dinamică diferenţială (DSC).

Keywords: repaglinide, inclusion complexes, cyclodextrins

Introduction

Cyclodextrins (CDs) are cyclic oligomers of six, seven or eight linked α – D- glucopyranose units, denoted α-, β- and γ-CDs, respectively.

The CDs are well known to form inclusion complexes with a variety of organic compounds, among them, with drug substances [10,11,13,14]. This ability is based on the capability of the CDs to provide a hydrophobic cavity in aqueous solution for the hydrophobic guest molecule or moieties in the guest molecule.

Studies involving inclusion of active pharmaceutical substances into CDs are important due to the resulting improvement of aqueous solubility [6, 12,17], stability of the guest molecule [1,3,4] and to the possibility of controlled drug release [4,15,16], which present many potential applications in drug formulations.

Repaglinide, 2-ethoxy-4-[2-[[3-methyl-1-[2-(piperidin-1-yl)phenyl] butyl]amino]-2-oxoethyl] benzoic acid (figure 1) is an antidiabetic agent from the class of glinides, used for the treatment of patients with type 2 diabetes mellitus. The substance is characterized by very low water solubility (34 μg/mL at 37°C) and a high lipophilicity (logP = 3.97) [5].

The purpose of this study is to evaluate the possibility of increasing the solubility of repaglinide through complexation with cyclodextrins: β CD, HP- β-CD and RAMEB. The following preparation methods have been used for obtaining inclusion complexes between repaglinide and cyclodextrins: the lyophilization, co-precipitation and kneading methods.

The resulted complexes were characterized by means of: Nuclear Magnetic Resonance (^{1}H-NMR, ^{13}C-NMR), Differential Scanning Calorimetry (DSC) and Infrared Spectroscopy (FT-IR).

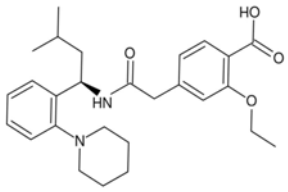

Figure 1. Repaglinide

Materials and methods

Materials

Repaglinide, with purity of 97.69% was obtained from Novo Nordisk A/S, Denmark.

β-cyclodextrin was purchased from Cyclolab (Hungary); 2-hydroxypropyl- β-cyclodextrin and the randomly methylated-β cyclodextrin (substitution rate 1.7-1.8) were obtained from Fluka (Sigma- Aldrich Chemie GmbH, Germany).

The solvent DMSO d6 was of spectroscopic NMR grade.

All the other reagents used were of analytical grade.

Methods

Preparation of the inclusion complexes

Lyophilisation:

1.5 mmol cyclodextrin/cyclodextrin derivative were dissolved in distilled water. A 50% ethanolic solution containing 0.75 mmol repaglinide was added stepwise to the aqueous solution of cyclodextrin/cyclodextrin derivative. The suspension was stirred for 6 hours in an ultrasonic bath and cooled for approx. 48 hours at 2-8°C and another 24 hours at -20°C and finally lyophilized at -60°C for 24 hours.

Co-precipitation:

The same preparation steps were followed as for the lyophlisation method; after stirring for 6 hours in an ultrasonic bath, the end-product was filtered through a G4 crucible and dried at 25°C in an exsiccator.

Kneading method:

1.5 mmol cyclodextrin/cyclodextrin derivative were weighed and brought in a mortar. A 50% ethanolic solution of repaglinide containing 0.75 mmol was prepared and added dropwise to the cyclodextrin powder, while triturating. After a mixing time of approx. 30 minutes, the end-product was dried in oven, for approx. 30 minutes at 40-60°C.

Apparatus

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 Bruker instrument, using DMSO-d6 for sample solubilization.

DSC measurements were performed on a DuPont Thermal Analyzer apparatus, using 40 μL Aluminium crucible with a heating rate of 20°C/min.

IR spectra were recorded on a FT-IR Bio-Rad FTS155 instrument, using the KBr sample preparation method.

Results and discussion

The most efficient preparation method for inclusion complexes has been proven to be the lyophilization method. Using the kneading method, a sticky paste, difficult to mix was formed, while the co-precipitation method is characterized by a lower reaction output, due to the filtration step. All the analysis presented in this paper have been performed on inclusion complexes between cyclodextrins and repaglinide, obtained through the lyophilization method.

A large variety of analytical methods can be successfully used to detect the formation of the inclusion complexes with CDs. Among them,

NMR technique offers detailed information on the equilibrium process and intramolecular changes [2, 8].

^{1}H-NMR and ^{13}C-NMR spectra

In the ^{1}H-NMR spectra of the formed inclusion compounds, repaglinide protons showed an upfield displacement due to a variation of local polarity and, also to the weak interactions with CD cavity hydrogen atoms.

Figures 2, 3 , 4 and 5 show the 1H-NMR spectra obtained for repaglinide and for the inclusion complexes: β CD-repaglinide, HP- β-CD -repaglinide and RAMEB-repaglinide, obtained through the lyophilisation method.

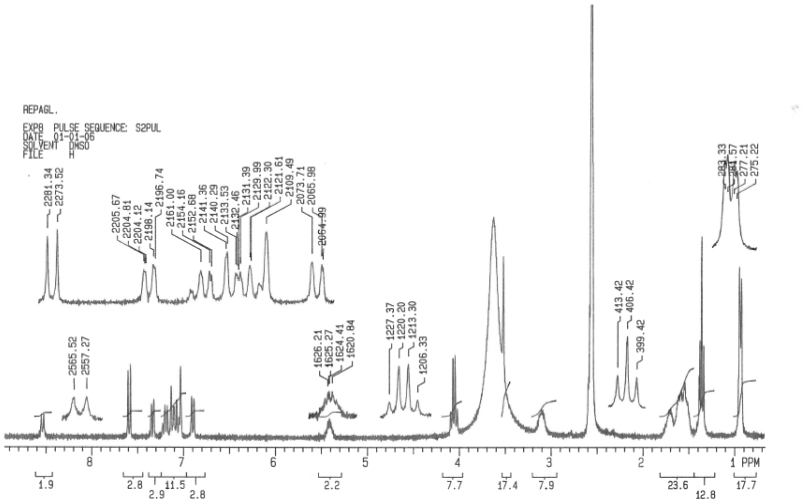

Figure 2. ^{1}H-NMR spectrum of repaglinide

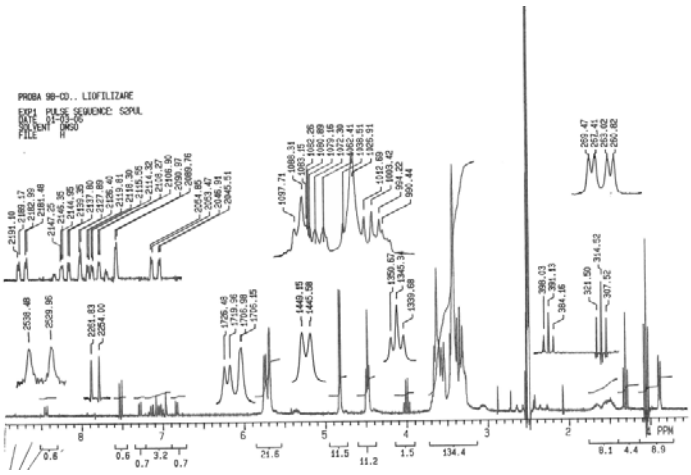

Figure 3. ^{1}H-NMR spectrum of β CD-repaglinide inclusion complex

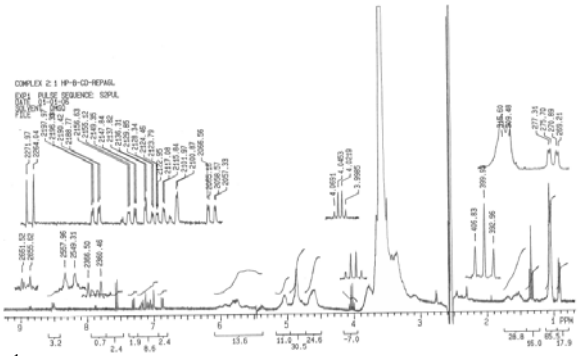

Figure 4. [1]H-NMR spectrum of HP-β-CD-repaglinide inclusion complex

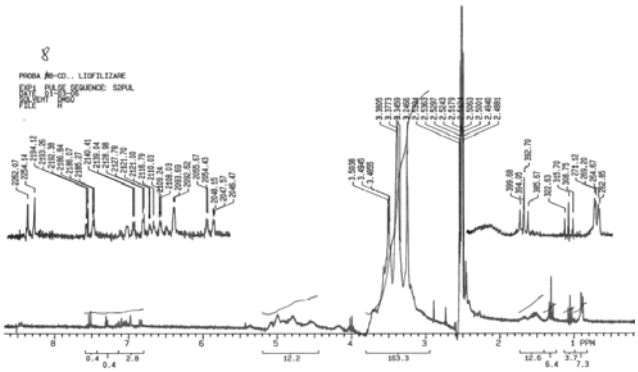

Figure 5. [1]H-NMR spectrum of RAMEB-repaglinide inclusion complex

In Table I the chemical shifts and their changes upon complexation for the repaglinide protons are presented, where $\Delta\delta = \delta_{complex} - \delta_{free\ repaglinide}$

Table I. Chemical shifts, δ (ppm) and chemical shifts variations for repaglinide protons

Repaglinide δ(ppm)	β-CD-repaglinide δ(ppm)	Δδ (ppm)	RAMEB-repaglinide δ(ppm)	Δδ (ppm)	HP- β-CD - repaglinide δ(ppm)	Δδ (ppm)
0.917	0.869	-0.048	0.876	-0.041	0.897	-0.020
0.924	0.877	-0.047	0.882	-0.042	0.903	-0.021
0.938	0.891	-0.047	0.897	-0.041	0.920	-0.018
0.944	0.899	-0.045	0.904	-0.040	0.924	-0.020
1.331	1.280	-0.051	1.285	-0.046	1.310	-0.021
1.355	1.304	-0.051	1.309	-0.046	1.333	-0.022
1.378	1.327	-0.051	1.332	-0.046	1.356	-0.022
6.883	6.823	-0.060	6.821	-0.062	6.858	-0.025
6.912	6.850	-0.062	6.852	-0.060	6.884	-0.028
7.032	6.970	-0.062	7.031	-0.001	7.003	-0.029
7.327	7.277	-0.050	7.289	-0.038	7.301	-0.026
7.352	7.304	-0.048	7.314	-0.038	7.326	-0.026
7.578	7.513	-0.065	7.514	-0.064	7.547	-0.031
7.604	7.539	-0.065	7.540	-0.064	7.573	-0.031

The results obtained from the ^{13}C-NMR analysis are summarized in table II.

Table II. Chemical shifts, δ (ppm) and chemical shifts variations for repaglinide C-atoms

Repaglinide δ(ppm)	β-CD-repaglinide δ(ppm)	Δ δ (ppm)	RAMEB-repaglinide δ(ppm)	Δ δ (ppm)	HP- β-CD -repaglinide δ(ppm)	Δδ (ppm)
15.528	14.524	-1.004	14.524	-1.004	15.513	-0.015
22.728	21.737	-0.991	21.739	-0.989	22.714	-0.014
24.133	23.097	-1.036	23.781	-0.352	24.122	-0.011
25.840	24.818	-1.022	24.825	-1.015	25.825	-0.015
27.293	26.265	-1.028	26.273	-1.020	27.259	-0.034
46.962	46.459	-0.503	46.471	-0.491	46.938	-0.024
64.966	64.031	-0.935	64.022	-0.944	66.306	1.340
121.750	120.756	-0.994	120.768	-0.982	121.736	-0.014
124.981	123.938	-1.043	123.935	-1.046	124.987	-0.006
126.930	125.956	-0.974	125.990	-0.940	126.911	-0.019
128.223	127.160	-1.063	127.144	-1.079	128.240	0.017
131.695	130.545	-1.150	130.519	-1.176	131.643	-0.052

The ^{1}H-NMR spectra of the inclusion complexes between repaglinide and different cyclodextrins showed significant changes in chemical shifts for the aliphatic (δ : 1.33 – 1.38 ppm; Δ δ: -0.051 ppm) and aromatic repaglinide protons (δ : 7.58 – 7.60 ppm; Δ δ: -0.065 ppm). These results suggest that the inclusion process involves mostly the hydrophobic part of repaglinide and the hydrophobic cavities of cyclodextrin.

The steric factor plays also an important role in the inclusion process, i.e. the highest variations of chemical shifts have been registered for the unsubstituted molecule of β CD, while the HP- β CD derivative, with the largest substituent, show the lowest changes in chemical shifts for the same repaglinide protons. 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. This observation is sustained also by the ^{13}C-NMR spectra: the most significant changes in chemical shifts for the carbon atoms of repaglinide are those for the aliphatic (δ: 24.13 – 25.84 ppm; Δ δ: -1.036-1.022 ppm) and aromatic parts (δ : 128.22 ppm; Δ δ: -1.063 ppm) of the drug structure. The magnitude of the variation of the chemical shifts changes in the order: β CD-repaglinide complex > RAMEB-repaglinide complex > HP- β-CD -repaglinide complex.

IR spectra

The IR spectrum of repaglinide reveals the presence of a peak at 3307.18 cm^{-1}, assigned to N-H stretching vibration and one at 1686.92 cm^{-1},

corresponding to the carbonyl group. The spectra of β CD, HP- β CD and RAMEB are characterized by intense bands at 3300-3500cm^{-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 [8]. Upon complexation, the repaglinide absorption peak at 3307.18 cm^{-1} was not identified any more in the IR spectra and the carbonyl band was slightly shifted towards a lower wavenumber, due to the appearance of host-guest interactions. These suggest the possibility of formation of hydrogen bonds between the hydroxyl groups of the host cavities and the repaglinide carbonyl group.

In figures 6, 7, 8 , 9, 10, 11 and 12 the IR spectra for: repaglinide, HP- β – CD, HP- β-CD –repaglinide, β-CD, β-CD –repaglinide, RAMEB and RAMEB-repaglinide are presented.

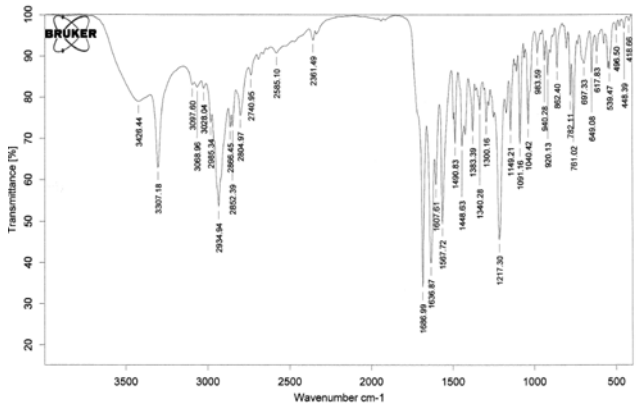

Figure 6. IR spectrum of repaglinide

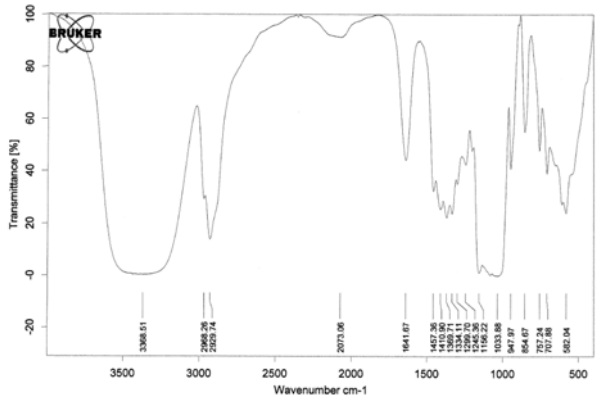

Figure 7. IR spectrum of HP- β CD

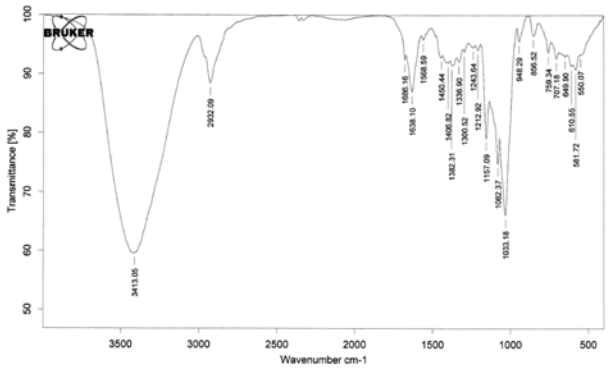

Figure 8. IR spectrum of HP-β-CD-repaglinide inclusion complex

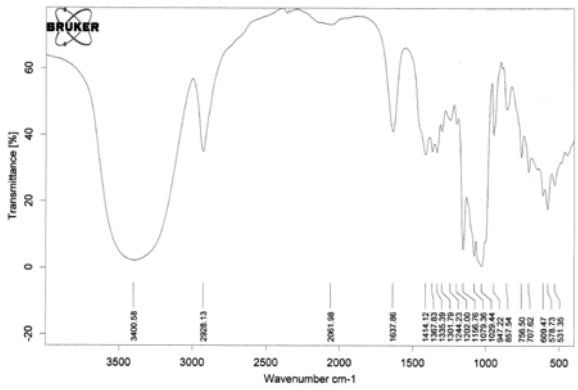

Figure 9. IR spectrum of β CD

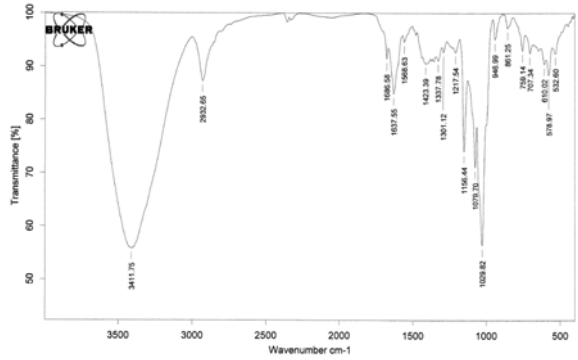

Figure 10. IR spectrum of β CD-repaglinide inclusion complex

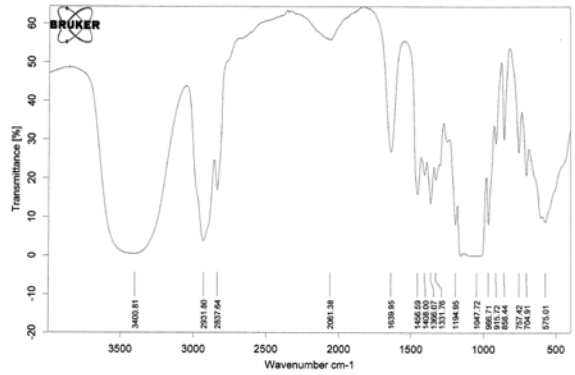

Figure 11. IR spectrum of RAMEB

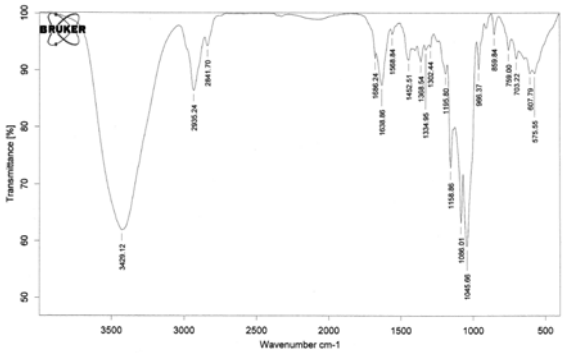

Figure 12. IR spectrum of RAMEB - repaglinide inclusion complex

Thermal analysis

The thermal analysis of repaglinide revealed a single, sharp endothermic peak at 138.26°C (melting point) (Figure 13), while the DSC curves for β CD, HP- β CD and methyl- β CD are characterized by broad endothermic effects, which attained a maximum around 100 - 120°C [7, 9]. An evidence for the formation of inclusion complexes between repaglinide and CDs was the change in the shape of the melting peak of repaglinide and its shift towards higher temperatures (e.g. for the complex HP- β CD: repaglinide the peak corresponding to the melting of the drug substance was observed around 141°C) (Figure 14), as a result of the inclusion of the drug into the cyclodextrins cavity.

Figures 13 and 14 show the DSC curves for repaglinide and HP- β-CD: repaglinide inclusion complex.

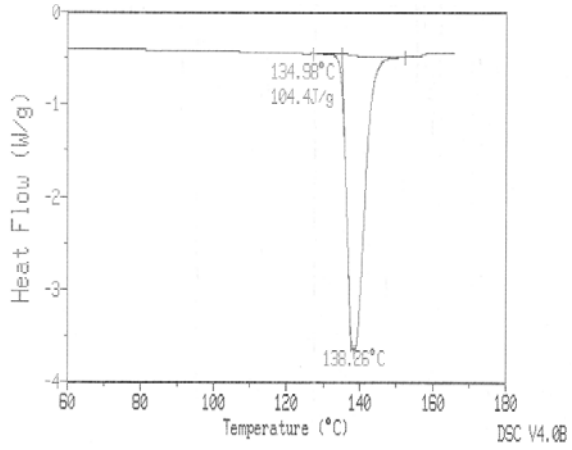

Figure 13. DSC curve of repaglinide

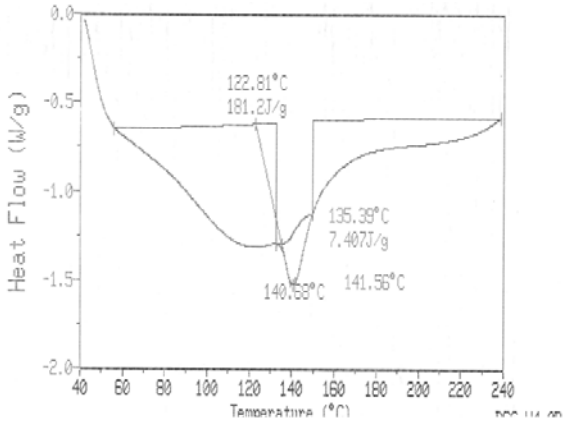

Figure 14. DSC curve of HP-β-CD-repaglinide inclusion complex

Conclusions

We consider the results obtained by NMR, IR Spectroscopy and Thermal Analysis to serve as proof for the formation of inclusion complexes between β CD, HP- β CD, RAMEB and repaglinide, respectively. Conclusive results have been obtained from the inclusion complexes prepared by the lyophilization method. From the cavity internal geometry point of view, the most favorable configuration for inclusion complex with repaglinide is that of β CD, while the formation of the HP-β-CD-repaglinide is hindered by the larger substituent volume.

The solubility data and the molecular ratios for the three inclusion complexes of repaglinide (with β CD, HP- β CD and RAMEB) are the subject of another paper.

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