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ORIGINAL ARTICLE

Rapamycin-cyclodextrin complexation: improved solubility and dissolution rate

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Abstract The objective of this study was to improve poor aqueous solubility and dissolution properties of anticancer drug rapamycin through formation of inclusion complexes with natural and modified cyclodextrins. Of the cyclodextrins tested, γ-cyclodextrin and hydroxypropyl-γ-cyclodextrin did not complex with rapamycin. However, complexes of rapamycin with β-cyclodextrin, methyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin were prepared and characterized by techniques such as Fourier Transform infrared spectroscopy, differential scanning calorimetry, phase solubility analysis and in vitro dissolution studies. According to the characterization data for the complexes, rapamycin water solubility was highly enhanced by all three β-cyclodextrins with methyl-β-cyclodextrin complex resulting in particularly higher solubility enhancement. FTIR spectra and DSC thermograms supported the formation of inclusion complexes. The complexes showed highly improved dissolution rate in water. Complexation with cyclodextrin derivatives such as methyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin can provide promising alternatives for the formulation of rapamycin.

Keywords Rapamycin · Sirolimus · Cyclodextrin · Dissolution

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Abbreviations

CD	Cyclodextrin
βCD	β-Cyclodextrin
MβCD	Methyl-β-cyclodextrin
HPβCD	Hydroxypropyl-β-cyclodextrin
γCD	γ-Cyclodextrin
HPγCD	Hydroxypropyl-γ-cyclodextrin
Rapa	Rapamycin

Introduction

Rapamycin also known as sirolimus is a complicated macrocyclic lactone, a white crystalline solid, Fig. 1. It is a lipophilic substance soluble in most organic solvents but virtually insoluble in water. Rapamycin was first isolated as an antifungal agent [1]. Shortly afterwards, it was found to have immunosuppressant [2–4] and antiproliferative/antitumor properties [5, 6].

Its oral bioavailability is reported to be only about 17% [7]. This is a potentially costly drawback for oral administration. Rapamune® was approved by FDA in 1999 for the prevention of organ rejection. Rapamycin is available as Rapamune® Oral Solution and Rapamune® Tablets. The systemic availability of sirolimus was estimated to be only approximately 14 and 17.78% after the administration of the solution and tablet, respectively [8]. These commercially available products are complex formulations of oral solution containing 1 mg/mL sirolimus and NanoCrystal® technology tablet containing 1 and 2 mg sirolimus. De et al. [9] used nanoparticle albumin-bound (nab™) technology to prepare nab-rapamycin system. Liposomal formulations [10, 11] were used to dissolve extremely

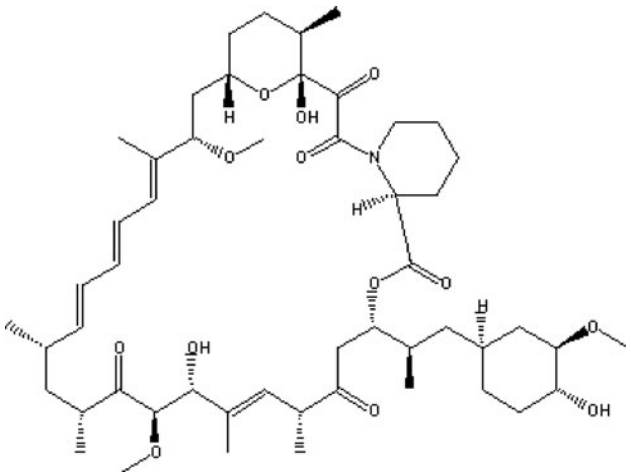

Fig. 1 Chemical structure of rapamycin

hydrophobic rapamycin, water solubility being 2.6 μg/mL [12]. Other rapamycin delivery systems reported were injectable formulation with amphiphilic block co-polymer [13], perfluorobutane gas microbubble carrier [14], and sirolimus-coated stents [15–19]. These are complicated and expensive technology based products.

Cyclodextrins (CDs) are cyclic molecules composed of glucopyranose ring units to form truncated cone type, doughnut structures [20]. The most common are the α, β, and γ-cyclodextrins which are composed of six, seven and eight sugar units, respectively. The exterior of the CDs are hydrophilic while the interior is hydrophobic in nature. Different CDs possess different cavity sizes according to the number of glucopyranose rings present. As a result of their unique ability to form inclusion complexes by incorporation of hydrophobic molecules or part of the molecules in their cavity, CDs provide a number of benefits in pharmaceutical formulations. They increase the water solubility of poorly soluble drugs to improve their dissolution profile and bioavailability, improve stability of active substances, and reduce dermal, gastrointestinal or ocular irritation [21].

The capability of rapamycin to complex with CDs and characterization of the complexes have not been reported earlier. One report mentioned rapamycin solution with hydroxypropyl-β-cyclodextrin (HPβCD) and hydroxypropyl-γ-cyclodextrin (HPγCD) for ocular delivery without characterization of the complexes [12]. Therefore, the objective of this study was to determine whether rapamycin complexes with various cyclodextrins to improve physicochemical properties of rapamycin, water solubility in particular.

Materials

Rapamycin (Sirolimus) was purchased from LC Laboratories, PKC Pharmaceuticals Inc., USA. Natural and modified cyclodextrins used in the study were as follows: β-cyclodextrin (βCD, Kleptose®, Roquette Freres, Lestrem, France), methyl-β-cyclodextrin (MβCD, CAVASOL® W7 M Pharma, Wacker Chemie AG, Germany, molar degree of substitution per anhydro glucose unit 1.7–1.9, average MW 1310), 2-hydroxypropyl-β-cyclodextrin (HPβCD, CAVASOL® W7 HP Pharma, Wacker Chemie AG, Germany, molar degree of substitution per anhydro glucose unit 0.58–0.73, average MW 1400), γ-cyclodextrin (γCD, Roquette Freres, Lestrem, France), and 2-hydroxypropyl-γ-cyclodextrin (HPγCD, CAVASOL® W8 HP PHARMA, Wacker Chemie AG, Germany, molar degree of substitution per anhydro glucose unit 0.5–0.7, average MW 1576).

Methods

Effect of cyclodextrins on rapamycin solubility

Rapamycin was first dissolved in ethanol in 5 separate vials, followed by evaporation of alcohol with nitrogen stream. Then, 1.14% w/v of βCD (10 mM), 1.30% w/v of γCD (10 mM), 5% w/v of HPβCD, 5% w/v MβCD, and 5% w/v HPγCD solutions, 10 mL each, were added to the above vials and magnetically stirred. Excess of rapamycin was taken in each vial so that a solid phase was always maintained. After 3 and 7 days, 2 mL cyclodextrin drug suspensions from each vials were filtered through 0.20 μm polycarbonate membrane filters fitted in a stainless still filter holder. The filtrates were then analyzed by HPLC.

Phase solubility analysis

Observing no effect of γCDs on the solubility of rapamycin, phase solubility analysis of rapamycin was performed as follows with βCDs: rapamycin was dissolved in ethanol in separate vials, followed by evaporation of alcohol with nitrogen stream. 0, 1, 2, 4, 6, 8, 10, 12 and 16 mM of βCD solutions; 0, 5, 10, 15, 20, 25, 30, 35, 40% w/v solutions of MβCD and HPβCD each, all in distilled water, were added to thirty separate vials. Excess of rapamycin was added to each vial so that a solid phase is always maintained throughout 7 days. After this period, 2 mL cyclodextrin drug suspensions from each of the vials were filtered through 0.20 μm polycarbonate membrane filter fitted in a stainless still filter holder. The filtrates, diluted with mobile phase if required, were then analyzed by HPLC. Phase solubility diagrams were prepared by plotting cyclodextrin concentration versus solubilized drug concentration. The apparent stability constants (K) were calculated for the rapamycin:CD complexes by the Connors Method [22] using the slope of the curve for 1:1 complex type phase

solubility by the following equation, where S_w is intrinsic solubility of rapamycin.

$$K = \frac{slope}{(1 - slope) * S_W}$$

Characterization of complexes

For the characterization of rapamycin:cyclodextrin complexes, complexation was performed at the cyclodextrin concentration near the maximum point in solubility diagram following the same procedure as described above. After complexation, filtered solutions of rapamycin:cyclodextrin complexes were lyophilized to remove water and to have the complexes in solid/powder forms.

Fourier transform infrared (FTIR) spectra of rapamycin, βCD, MβCD, HPβCD, rapamycin:βCD complex, rapamycin:MβCD complex and rapamycin:HPβCD complex were taken between the wave number of 400 and 4000 cm^{-1} with a Nicolet 520 FTIR spectrometer (Thermo Electron Corp, Waltham, MA) using previously prepared discs of 0.01 g of each sample and 0.1 g of potassium bromide.

Differential scanning calorimetry (DSC) analyses were performed on rapamycin, βCD, MβCD, HPβCD, rapamycin:βCD complex, rapamycin:MβCD complex and rapamycin:HPβCD complex samples with a DuPont 910 differential scanning calorimeter (Wilmington, DE). Each sample weighing ~3 mg was heated in hermetically sealed aluminum pans at a rate of 10 °C/min up to 200 °C in a dynamic nitrogen atmosphere.

The dissolution rates of rapamycin complexes with natural and modified cyclodextrins were measured by the dispersed/dissolved amount method [23]. Dissolution experiments were carried out with a Sotax dissolution test apparatus in distilled water at 37 °C by the paddle method at a rotation speed of 60 rpm [24]. Rapamycin alone and three rapamycin:cyclodextrin complexes were included in this study. Each powder sample equivalent to 75 μg of rapamycin was added to 900 mL of distilled water as dissolution medium. At appropriate time intervals, 2 mL samples were withdrawn and filtered through a 0.20 μm polycarbonate filter. The initial volume of dissolution medium was maintained by adding 2 mL of fresh medium. The filtrates were analyzed by HPLC.

HPLC methods developed by Ferron et al. [25] and Holt et al. [26] to analyze rapamycin in biological samples was modified and used for the quantification of rapamycin. The conditions used for HPLC analysis are as follows; HP Agilent 1100 Series, UK equipped with reverse phase column: μBondapack C18, 10 μm 125A, 3.9 × 300 mm, autosampler: Thermostat set at 20 °C, Column Oven Temperature (analysis temperature): 50 °C, pump: set to deliver 0.75 mL/min, Diode Array Detector: set at 278 nm, Data Acquisition System: Agilent ChemStation LC 3D Rev.01.01 164, Isocratic Mobile Phase: 80:20 (v/v) mixture of methanol and water, injection volume: 100 μl.

Statistical analysis of data was performed by statistical software program, *STATISTICA* Kernel Release 5.5 (StatSoft, Inc, USA). Statistical significance was considered at $P < 0.05$.

Results and discussion

Effect of cyclodextrins on rapamycin solubility

To see whether rapamycin complexes with cyclodextrins at all, 10 mM βCD and 10 mM γCD (1.14 and 1.30% w/v, respectively); and 5% w/v each of MβCD, HPβCD and HPγCD were stirred with rapamycin. Rapamycin solubilities with βCD, γCD, MβCD, HPβCD and HPγCD are given in Table 1. HPLC analysis after 3 and 7 days showed that there was a very little difference between the solubilities after 3 and 7 days of mixing. This initial experiment showed that βCD, MβCD and HPβCD increased rapamycin solubility in water to a greater extent. Gamma-CD and HPγCD did not increase the solubility. For this reason, further studies were performed only with βCD, MβCD and HPβCD. Table 2 Shows the increase in rapamycin solubility with various concentrations of these three CDs.

The solubility of rapamycin was reported to be only 2.6 μg/mL by previous studies [12]. One of the methods to increase solubility of lipophilic drugs is complexation with cyclodextrin. Rapamycin crystals are very hard and float on water minimizing drug-cyclodextrin molecular interaction. Increased solubility of rapamycin is detectable only if the drug is dissolved in ethanol and evaporated to dryness before CDs and water are added. Dissolving in alcohol and drying with nitrogen stream produced a rapamycin film, amorphous form of rapamycin, which remained suspended during stirring. Crystalline rapamycin clearly seems to be more hydrophobic than amorphous form. Beta-CD, MβCD

Table 1 Solubilities of rapamycin with various cyclodextrins

CDs	%w/v	After 3 days (μg/mL)	After 7 days (μg/mL)
Only RAPA	–	3.78	3.80
βCD	1.14	18.59	17.64
MβCD	5	219.39	232.16
HPβCD	5	37.54	41.89
γCD	1.30	2.95	2.11
HPγCD	5	3.52	3.49

CDs cyclodextrins, *RAPA* rapamycin, *βCD* β-cyclodextrin, *MβCD* methyl-β-cyclodextrin, *HPβCD* hydroxypropyl-β-cyclodextrin, *γCD* γ-cyclodextrin, *HPγCD* hydroxypropyl-γ-cyclodextrin

Table 2 Solubilities of rapamycin in βCD, MβCD and HPβCD solutions

Cyclodextrins	Cyclodextrin concentration, %w/v	Rapamycin Solubility (μg/mL ± SD, $n = 3$)	Increase in Solubility[a]
βCD	0	3.80 ± 1.38	–
βCD	0.11	5.43 ± 1.38	1.43
βCD	0.23	6.44 ± 0.91	1.70
βCD	0.45	8.33 ± 0.61	2.19
βCD	0.68	9.94 ± 1.57	2.62
βCD	0.91	13.28 ± 0.56	3.50
βCD	1.14	16.82 ± 1.75	4.43
βCD	1.36	18.03 ± 1.77	4.75
βCD	1.82	20.25 ± 1.60	5.33
$Y = 0.0013\ X + 4 \times 10^{-6}$, $R^2 = 0.9735$; $K = 313\ M^{-1}$			
MβCD	0	3.80 ± 1.38	–
MβCD	5	229.10 ± 40.31	60.29
MβCD	10	350.66 ± 30.91	92.28
MβCD	15	425.68 ± 24.05	112.02
MβCD	20	531.84 ± 54.73	139.96
MβCD	25	711.39 ± 29.68	187.21
MβCD	30	807.86 ± 47.86	212.59
MβCD	35	869.33 ± 67.32	228.77
MβCD	40	1029.37 ± 108.42	270.89
$Y = 0.0038\ X + 4 \times 10^{-6}$, $R^2 = 0.9738$; $K = 917\ M^{-1}$			
HPβCD	0	3.80 ± 1.38	–
HPβCD	5	43.37 ± 11.74	11.41
HPβCD	10	97.76 ± 19.94	25.73
HPβCD	15	148.05 ± 22.27	38.96
HPβCD	20	224.40 ± 18.69	59.05
HPβCD	25	297.44 ± 46.84	78.27
HPβCD	30	543.53 ± 35.33	143.04
HPβCD	35	600.95 ± 30.98	158.14
HPβCD	40	620.41 ± 36.24	163.27
$Y = 0.0024\ X + 4 \times 10^{-6}$, $R^2 = 0.9213$; $K = 579\ M^{-1}$			

βCD β-cyclodextrin, *MβCD* methyl-β-cyclodextrin, *HPβCD* hydroxypropyl-β-cyclodextrin

[a] Solubility of rapamycin with cyclodextrins divided by solubility with no cyclodextrin

and HPβCD, particularly MβCD, increased rapamycin solubility to a great extent. As γCD and HPγCD did not increase the solubility of rapamycin, they were excluded from the subsequent experiments. The reason for lack of effect by these two CDs is probably their larger cavity size, allowing no good fit between drug and CDs. Though solubilities of rapamycin with CDs after 3 and 7 days of mixing were very close to each other, all the subsequent experiments were performed for 7 days to be sure about the equilibrium.

Corresponding solubility diagrams of rapamycin with βCD, MβCD and HPβCD are presented in Fig. 2 and Fig. 3. These solubility diagrams seem to be of the A_L type (linear increases of drug solubility as a function of CD concentration). From the phase solubility diagrams, it can be said that the solubility of rapamycin increases linearly with increasing concentration of various CDs. Solubility of rapamycin in μg/mL as well as increase in solubility compared to the intrinsic solubility, corresponding correlation coefficients (R^2) and corresponding apparent stability constants (K) are presented in Table 2.

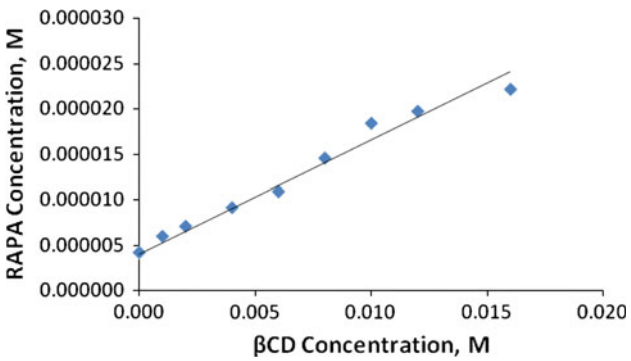

Fig. 2 Phase solubility diagram of rapamycin with β-cyclodextrin in distilled water ($n = 3$)

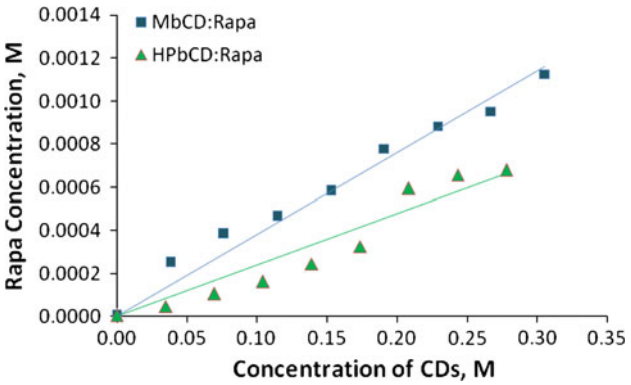

Fig. 3 Phase solubility diagrams of rapamycin with methyl-β-cyclodextrin and hydroxypropyl -β-cyclodextrin in distilled water ($n = 3$)

Beta-CD increased rapamycin solubility to a modest extent because of its own limited solubility. At 1.8% w/v concentration of βCD, its approximate solubility limit in water, the solubility of rapamycin increased by approximately fivefold compared to that in water alone in which rapamycin was treated in the same way as with various CD concentrations. Of the modified βCDs used in the study, MβCD solubilized rapamycin to the greatest extent. At 40% w/v concentrations, MβCD and HPβCD increased the solubility of rapamycin by factors of 270 and 163 respectively, compared to the solubility value obtained in water alone, i. e. without any CDs. However, 40% w/v solutions of these CDs are slightly viscous. The 25–30% w/v concentration of MβCD, which is practically not more viscous than water, increases rapamycin solubility by factors of 187–212 producing rapamycin solution in the range of 0.70–0.80 mg/mL. The 25–30% w/v concentration of HPβCD, which is also practically not more viscous than water, increases rapamycin solubility by factors of 78–143 producing rapamycin solution of 0.29–0.54 mg/mL.

These solubility study results suggest that MβCD is a more effective solubilizing agent for rapamycin. It was also reported in the published literature [21] that among the CDs or modified CDs, methyl derivatives are the most powerful solubilizing agents. This was the case with rapamycin also. The higher solubilizing effect of MβCD compared to other CDs can be attributed to the methyl groups, which not only disrupt intramolecular hydrogen bonding, making the MβCD molecule highly soluble in water, but also enlarge the whole cavity of the molecule by extending the secondary hydroxyl side and narrowing the primary hydroxyl side of the cone [27].

The 1:1 stability constants (K M^{-1}) for various rapamycin:CD complexes were determined by the phase-solubility method. The strongest stability constant was found for rapamycin:MβCD complex (K = 917 M^{-1}) among the three complexes studied. This explains the reasons for higher solubility of rapamycin with MβCD. The stability constants for rapamycin:βCD and rapamycin:HPβCD complexes were 313 and 579 M^{-1}, respectively, also indicating a strong stability constant for the complexes and high affinity of rapamycin for the CD cavities.

Characterization of rapamycin:CD complexes by FTIR

FTIR spectra of rapamycin, individual cyclodextrins (βCD, MβCD, HPβCD) and rapamycin complexes with these 3 CDs were compared (spectra not shown). It was observed that characteristic C=C stretch band (1680–1640 $cm^{-1)}$ and C=O stretch band (1760–1670 cm^{-1}) in the rapamycin spectrum disappeared in the spectrum of rapamycin:βCD complex. Another change noticed in the spectrum of rapamycin:βCD complex was the disappearance of alkenyl C–H stretch at 3080–3020 cm^{-1} adjacent to alkyl C–H stretch at 2960–2850 cm^{-1}. These C=C, C=O and alkenyl C–H groups are not present in the structures of cyclodextrins used.

Similar observations were found in the cases of rapamycin:MβCD and rapamycin:HPβCD complexes. Characteristic rapamycin C=C stretch band (1680–1640 cm^{-1}), C=O stretch band (1760–1670 cm^{-1}) and alkenyl C–H stretch (3080–3020 cm^{-1}) in the rapamycin spectrum disappeared in the spectra of rapamycin:MβCD complex and rapamycin:HPβCD complex.

FTIR study investigated the functional groups of rapamycin involved in the complexation. Liu et al. reviewed the forces involved in the inclusion complexation of cyclodextrins [28]. The electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding, and charge–transfer interaction were the forces suggested to be involved in the inclusion complexation of cyclodextrins. Release of conformational strain and exclusion of cavity-bound high-energy water were ruled out as energetically contributive forces to the complex formation due to enthalpy–entropy compensation. Driving forces important in particular complexation systems could be predicted by the analyses of multivariate quantitative structure–activity relationship. Such analysis was beyond the scope of our study. The disappearance of characteristic rapamycin bands (C=C, C=O and alkenyl C–H stretch bands) in the spectra of rapamycin:βCD, rapamycin:MβCD and rapamycin:HPβCD complexes indicated the formation of cyclodextrin-rapamycin complexes. Otero-Espinar et al. [29] reported that the carbonyl group (e.g., in rapamycin) is affected by hydroxylic compound (e.g., cyclodextrins) due to the formation of hydrogen bonds. In another structural investigation of inclusion complex of vitamin B13 (orotic acid) with β-cyclodextrin,

strongly diminished intensity of C=O and C=C stretching band after complexation with β-CD was related with the ring mobility hindering and hydrogen bonds during the complexation process [30]. While one or more than one of the above mentioned forces might be involved, we assume, in light of the above discussion, that hydrogen bonding is the most likely reason for changes on rapamycin bands while in the complex state.

Characterization of rapamycin:CD complexes by DSC

The DSC thermograms of rapamycin, corresponding cyclodextrin and rapamycin:cyclodextrin complexes are presented, in groups, in Figs. 4, 5, and Fig. 6. Rapamycin showed an endothermic peak in the region of 183–185 °C corresponding to its melting point. The split peak is probably related to the two different crystal forms of

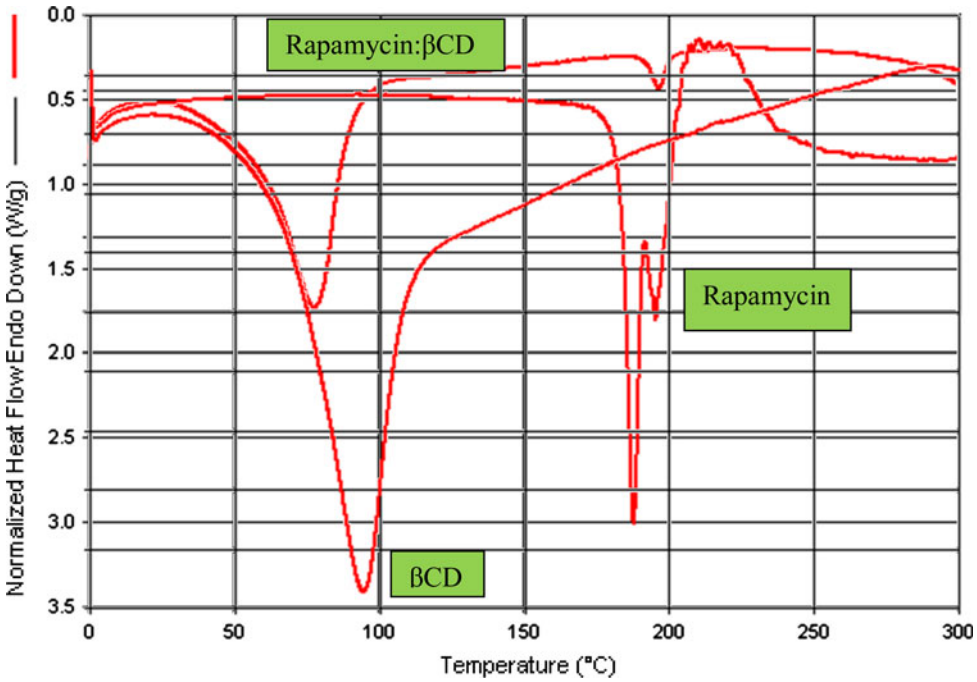

Fig. 4 Thermograms of rapamycin, βCD and rapamycin:βCD complex

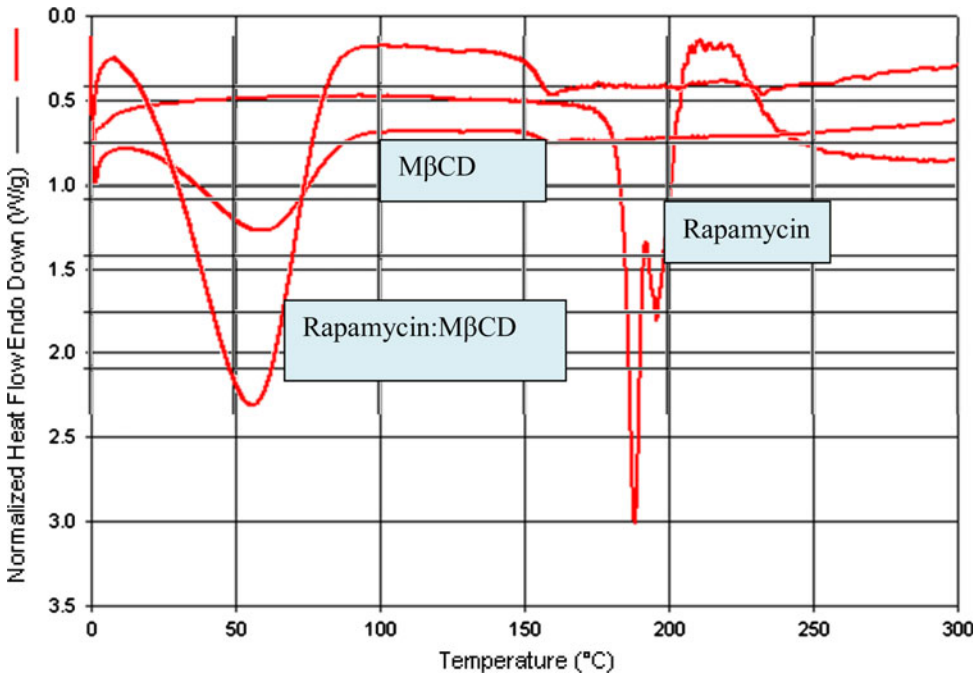

Fig. 5 Thermograms of rapamycin, MβCD and rapamycin:MβCD complex

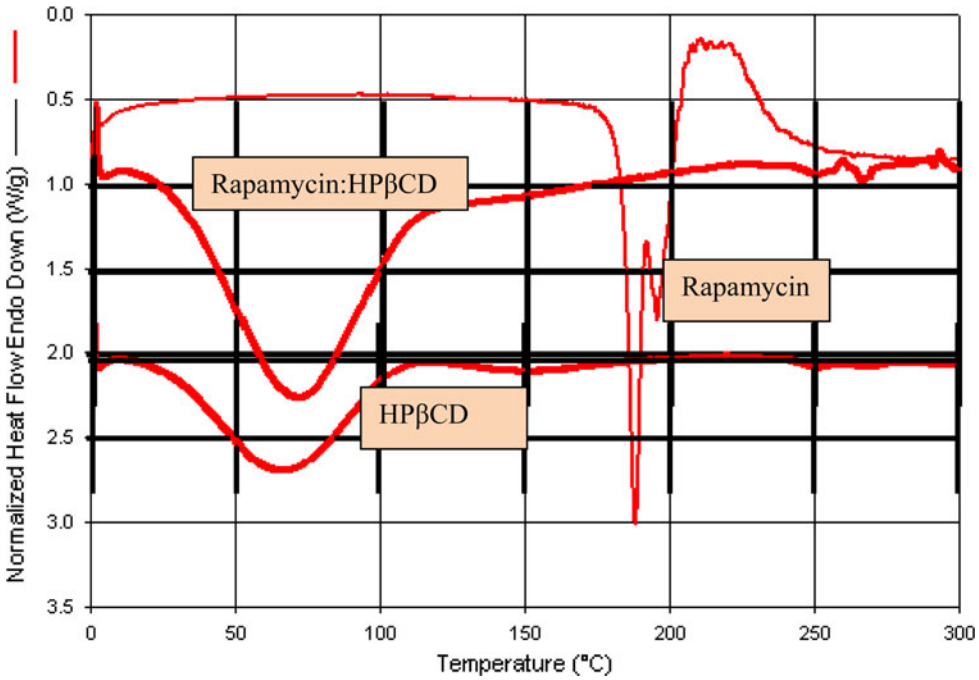

Fig. 6 Thermograms of rapamycin, HPβCD and rapamycin:HPβCD complex

rapamycin seen in microscopic observation. The thermogram of βCD and HPβCD showed a very broad endothermic effect which is attributed to the release of water molecule [31]. This may be the case for MβCD also as it also showed broad endothermic peak in the same temperature region. Rapamycin melting point peak has become very short in the thermogram of rapamycin:βCD complex whereas it has completely disappeared in the thermograms of rapamycin:MβCD and rapamycin:HPβCD complexes.

The dehydration peaks of the cyclodextrins seem to have been affected by complexation. The thermograms show the normalized heat flow for each thermogram. Relative amount of energy consumed by the pure CDs and respective rapamycin:CD complexes for the dehydration peak are markedly different.

Thermal analysis of rapamycin, various cyclodextrins and their complexes show indications of inclusion complexation. When guest molecules or part of the molecules are embedded in CD cavities or in the crystal lattice, their melting, boiling or sublimation points generally shift to a different temperature or disappear within the temperature range where CD is decomposed [32]. This has happened with all of the three rapamycin:CD complexes studied. The interaction between CDs and rapamycin is clear from the complete disappearance (rapamycin:MβCD and rapamycin:HPβCD) or shortening (rapamycin:βCD) of rapamycin melting point peak and changes in the dehydration peaks of pure cyclodextrins in the DSC thermograms of the complexes.

Characterization of rapamycin:CD complexes by dissolution test

The rate of dissolution of pure rapamycin and three rapamycin:CD complexes can be observed from the dissolution profiles as presented in Fig. 7. The amount of rapamycin used in dissolution experiment was 75 µg in 900 mL distilled water for each samples. The pure rapamycin dissolution rate is very slow, with 25% of the drug being dissolved after 1 h; less than 60% of the drug was dissolved after two and a half hour. In contrast, the dissolution of complexes is more than 80% within 10 min.

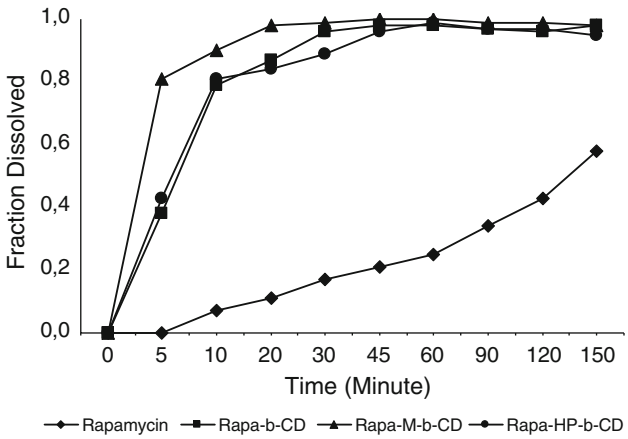

Fig. 7 Dissolution profile of rapamycin, rapamycin:βCD complex, rapamycin:MβCD complex and rapamycin:HPβCD complex in distilled water

High rate of dissolution of rapamycin as CD complexes can be seen in Fig. 7. The reported solubility of rapamycin is 2.6 μg/mL and our determined solubility of alcohol treated rapamycin is 3.8 ± 1.8 μg/mL, as determined during phase solubility analysis. Considering these solubility values, 900 mL medium for 75 μg rapamycin sufficiently provide sink condition as 75 μg was extremely less than one-third of the amount that can be dissolved on saturation. The pure rapamycin dissolution rate is very slow compared to those of rapamycin complexes. High rate of dissolution by cyclodextrin complexes of lipophilic drug was reported before [24]. Decrease in crystallization tendency and increase in solubility of the active substance were suggested as the mechanism of increase in dissolution rate by CD complexation [33].

Conclusion

In the light of the data in this study, it can be concluded that complexation of rapamycin with cyclodextrin derivatives such as methyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin can provide promising alternatives for the formulation of rapamycin. Methyl-β-cyclodextrin can provide an effective and simple oral preparation of rapamycin with increased aqueous solubility and fast dissolution rate. Therefore, MβCD complexation is a promising approach to improve the poor oral bioavailability of rapamycin. However, MβCD is not suitable for parenteral use because of its hemolytic effect. Instead, rapamycin:HPβCD can be considered for intravenous route of application as HPβCD has no such problematic effect. Improved solubility and dissolution rate should enhance gastrointestinal absorption and anti-cancer effect of rapamycin. These studies are in progress with cell culture method using caco-2 and MCF-7 cells.

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