Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Comparison the solubility and dissolution rate effects of beta-cyclodextrin and gamma-cyclodextrin complexations of rosuvastatin calcium

Dilara ÖRGÜL [1] *

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy, Selcuk University, Konya, Turkey.
* Corresponding Author. E-mail: dilara.orgul@selcuk.edu.tr, dilaraorgul@gmail.com (D.O.); Tel. +09-332-223 32 07; ORCID No: 0000-0002-8947-4995

Received: 14 August 2023 / Revised: 19 September 2023 / Accepted: 21 September 2023

ABSTRACT: The present work focuses on the inclusion complexations (ICs) of rosuvastatin calcium (RSV) with beta (β) ve gama (γ) derivatives of native cyclodextrins (CDs) and investigate effects on improved solubility and dissolution rate. The phase solubility studies illustrated that RSV solubility increased in the presence of γCD with a negative deviation that indicates A_N type diagrams, while βCD showed Bs type, upon addition of βCD, an increase in the solubility of the drug was observed up to a particular point. ICs of RSV were prepared with β and γCD by at 1:1, 1:2 and 1:4 different molar ratios by freeze drying method. FT-IR and DSC results revealed formation of ICs between RSV and CD. High drug loading efficiency was obtained for all ICs in the range of 99.41–101.84%. Water solubility studies showed that βCD ICs have increased solubility of RSV about 1.3 times regardless of CD ratios($p>0.05$). As compared to βCD ICs, RSV solubility was significantly greater in γCD ICs and increased up to 1.45, 1.72 and 2.00 fold at 1:1, 1:2 and 1:4 ratio, respectively. Conspicuously, RSV solubility increased with increasing ratio of γCD($p<0.05$). Compared dissolution profiles with pure RSV, all ICs showed improved dissolution rates and immediate release profiles. However multiple point comparison of dissolution profiles indicated that γCD ICs have higher drug release. Particularly γCD ICs at 1:4 ratio released the highest RSV with 95.12% at 3 min and 100% completion in 15 min. It is concluded that γCD provided a better improvement on RSV solubility and dissolution rate than βCD.

KEYWORDS: Rosuvastatin calcium; beta cyclodextrin; gama cyclodextrin; inclusion complexation; dissolution rate; solubility.

1. INTRODUCTION

An orally administered drug must first be dissolved in the gastrointestinal fluids to reach into the bloodstream and sites of action. In the absence of sufficient solubility, drugs show poor and/or variable absorption, tissue distribution, and also metabolic behavior [1]. In particular, poor aqueous solubility cause to low and variable oral bioavailability in Biopharmaceutical Classification System (BCS) class II drugs, which have low solubility and high permeability [2]. Currently, poorly soluble drugs represent approximately 40% of the top 200 oral drugs marketed in the USA and Europe. Therefore, aqueous solubility is one of the most critical and important parameters in the bioavailability improvement for orally administered pharmaceutical products. Also, leading healthcare organizations such as the World Health Organization, the United States (US) Food and Drug Administration (FDA), and the The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) give recommendations for reducing adverse effects risks of drugs by enhancing solubility and stability, and ultimately improving the bioavailability. Because the considerable increase in drug bioavailability can be used to reduce the dose of the drug [2, 3].

Rosuvastatin calcium (RSV) is an orally administered synthetic HMG CO-A reductase inhibitors (statins). It was approved by FDA in August 2003 for the treatment of hyperlipidemia. RSV has a higher affinity for the enzyme's active site and more binding interactions with HMG-CoA reductase than other statin drugs. Studies have demonstrated that RSV was more effective in lowering LDL-C levels and increasing HDL-C levels compared with atorvastatin, simvastatin and pravastatin. For this reason, it represented a progress in the pharmacological and clinical efficacy of statins and used more widely than

How to cite this article: Örgül D. Comparison the solubility and dissolution rate effects of beta-cyclodextrin and gamma-cyclodextrin complexations of rosuvastatin calcium. J Res Pharm. 2024; 28(3): 797-807.

© 2024 Marmara University Press
ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.740

Journal of Research in Pharmacy
Research Article

other statin group drugs [4, 5]. However, RSV belongs to BCS class II with low solubility and high permeability. Due to its poor solubility in gastrointestinal fluids, RSV has limited oral dissolution and absorption rates. Moreover, after oral administration, RSV is extensively metabolized in the liver via oxidation, glucuronidation and lactonization reactions. In terms of stability, while the shelf life of tablets is 3 years, it is only stable for 30 days in suspension form. Therefore intensive first pass metabolism combined with its low solubility limits oral bioavailability, about 20% and therapeutic efficacy of RSV [6, 7].

In today's pharmaceutical R&D studies, solubility problems could overcome with formulation studies and significant improvements can be achieved in the clinical response of the drug. In order to increase the solubility and oral bioavailability of poorly soluble drugs in the gastrointestinal tract (GIT) physiological conditions, various methods are applied, such as the development of salt formulations of acidic or basic drugs, reduction of drug particle size, preparation of amorphous solid dispersions, polymorphic form modifications and preparation of water-soluble complexes [8-11]. Among these methods, cyclodextrins (CDs) inclusion complexations (ICs) have an important role in improving the solubility and subsequently bioavailability of active ingredients. Therefore, they are widely used as drug carriers in various administration routes such as oral and local administration to solubilize insoluble drugs and increase their bioavailability [12]. While the free hydroxyl groups outside the cyclodextrins give a hydrophilic character, the oxygen atoms and hydrogen atoms in the glycosidic bonds in the cyclodextrin molecule form a hydrophobic cavity. The hyphrophilic outer surface of cyclodextrins allows the poorly soluble pharmaceuticals that form an inclusion complex with the hydrophobic structure in the cavity, to dissolve in the aqueous medium [13, 14]. Beside improved solubility with CD complexation of pharmaceuticals, numerous improved effects of CDs have been reported such as increased permeability due to the interaction with biological membranes, enhanced dissolution rate and profile, masking of undesirable properties, protection from interactions in the physiological environment and prolongation of stability and shelf life [15, 16]. Due to their voluminous and hydrophilic nature, CDs are insignificantly absorbed from the gastrointestinal tract thus they are practically nontoxic in oral administration [13]. Thus, cyclodextrins are on the Generally Recognized as Safe (GRAS) list of the FDA. In addition, primary pharmacopoeias, including the European Pharmacopoeia (Ph.Eur.), the US Pharmacopoeia/National Formulary (USP/NF), and the Japanese Pharmacopoeia (JPC) contain monographs of CDs [14]. Taken together, CDs are considered to be an ideal drug carrier that meets current requirements in oral drug administration.

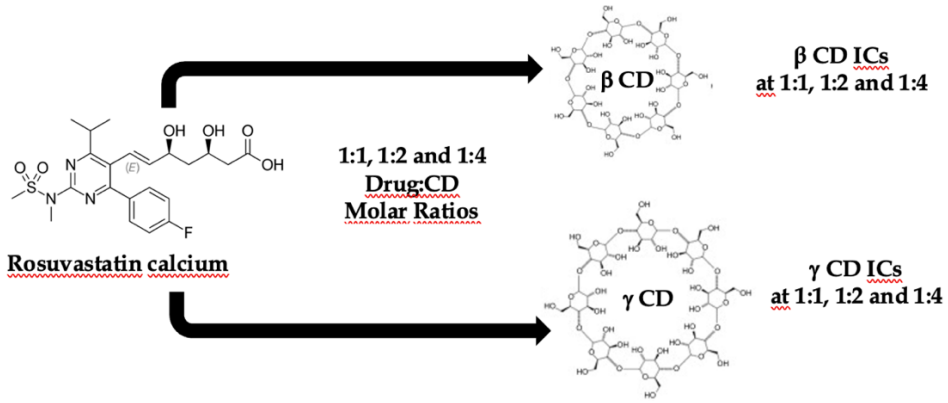

Figure 1. Chemical structures of of RSV, β and γ CD, and the illustration of inclusion complexations of RSV with β CD and γ CD at different drug:CD molar ratios of 1:1, 1:2 and 1:4.

In this context, the aim of present work was to prepare the inclusion complexations of RSV with beta (β) ve gama (γ) derivatives of native cyclodextrins and investigate effects on improved solubility and dissolution rate. After phase solubility studies, ICs of RSV were formed with β CD and γ CD at different molar ratios of 1:1,1:2 and 1:4 by using freeze drying method (Figure 1). Physicochemical characterization of the ICs were performed by fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) analysis. Complexatation, solubility and dissolution rate was evaluated based on drug loading efficacy, water solubility and in vitro dissolution analyses.

http://dx.doi.org/10.29228/jrp.740
J Res Pharm 2024; 28(3): 797-807

2. RESULTS AND DISCUSSION

2.1. Phase Solubility Studies

The phase solubility diagram of RSV in aqueous solution was generated by plotting the dissolved RSV concentration (mM) against the CD concentration (mM) (Figure 2). Plotting drug solubility versus cyclodextrin concentration is often used for determination of stoichiometry and apparent stability of inclusion complexes. Higuchi and Connors analyzed the phase solubility diagrams and classified the relationship between solubilizer concentration and the solubility of a substrate into two main types as A and B types [17, 18]. According to Higuchi and Connors, the phase solubility profile of γ CD illustrated that RSV solubility increased in the presence of γ CD with a negative deviation from linearity that indicates A_N type phase solubility diagrams [18]. A type profiles indicate that water-soluble complexes are formed with higher solubility than the uncomplex substrate. Otherside, β CD showed Bs type relation, upon the addition of β CD, an increase in the solubility of the drug was observed up to a particular point in 6 mM due to the lower aqueous solubility of β CD [12]. Type B phase-solubility profile of β-cyclodextrin show limited water-soluble complex formation. This result is in agreement with other inclusion complexes where β-cyclodextrin has been reported to be bound to hydrophobic drug molecules [19].

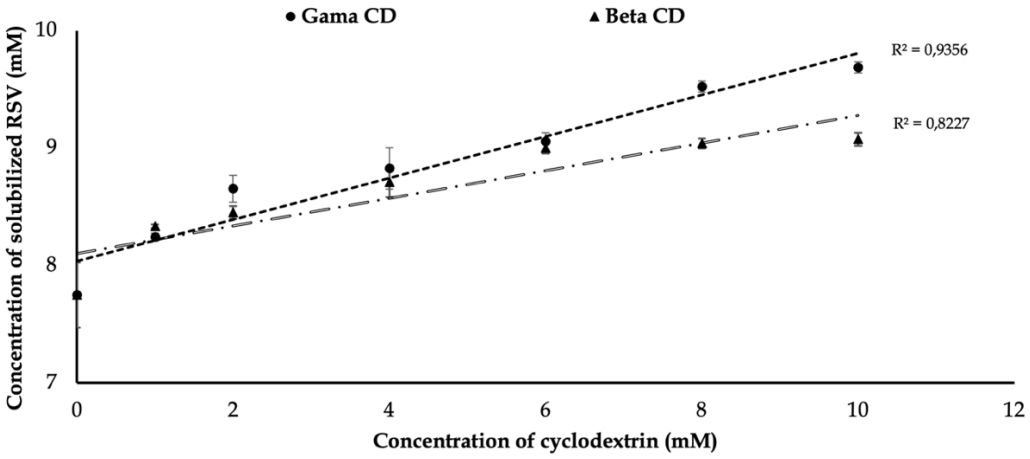

Figure 2. Phase solubility diagram of RSV in aqueous solution with β CD and γ CD (mean ± SD, n = 3)

Therefore, the 1:1 molar ratio of both Gamma and βCD inclusion complex were observed from the initial ascending part of curves. As shown in Figure 2, the saturation solubility of RSV increased to 9 mM and 9.69 mM with βCD and gamma CD, respectively. Generally, water soluble cyclodextrins form A-type phase solubility profiles, while less soluble cyclodextrins form B-type, so differences of phase solubility profile and saturation solubility observed between β CD and γ CD is thought to be due to the comparatively higher aqueous solubility of γ CD than β CD [17]. The apparent stability constants (Ks) calculated from the Equation 1, R^2 and the solubility values are given in Table 1. RSV in γ CD solution gave relatively higher solubility and more linear host guest correlation than β-CD. Ks values, was found to be 70.2 M^{-1} for β CD and 106.8 M^{-1} for γ CD that expressed drug's affinity for a certain cyclodextrin. Both K values were in range from 50 to 5000 M^{-1} therefore, ICs were considered suitable for solubility and stability improvements [20].

Table 1. Phase solubility parameters of the β CD and γ CD

Cyclodextrin	S_0 (mM)	Stability Constant Ks	Saturation Solubility (CD concentration) mM	R^2
β CD	7.45	70.2	9.00 (6 mM)	0.8227
γ CD		106.8	9.69 (10 mM)	0.9356

2.2. Physicochemical Characterization

2.2.1. Differential Scanning Calorimetry (DSC) analysis

Thermal behaviors of the ICs, the pure drug and CDs were investigated by DSC analysis to check the complexation. When pure drug were successfully embedded in the cavity of CDs, characteristic peaks of the pure drug disappear, broaden and/or shift to different temperatures.[21]. Obtained DSC-thermograms grouped for β and γ CDs are shown in Figure 3. The DSC-thermogram of RSV exhibited a short endothermic melting peak at approximately 140 °C as reported in the literature [22]. Before the melting point, an endothermic process is also observed from 90 °C to 100 °C, which represents water losses. The absence of a well-defined thermal peak versus melting point confirms the amorphous solid form of RSV. Lastly, a thermal event associated with the thermal degradation of amorphous form of RSV is observed in the range of 148-152 °C [23]. β ve γ CD thermograms showed the a sharp endothermic water loss peak. However, in the physical mixtures thermograms of these CDs with RSV, the presence of sharp water loss peaks of CDs is observed, while the characteristic thermal peaks of the amorphous structure of RSV slightly decrease and/or shift to different temperatures. This result is thought to be due to the weak interaction between RSV and CDs promoted by the heating process in the DSC analysis.

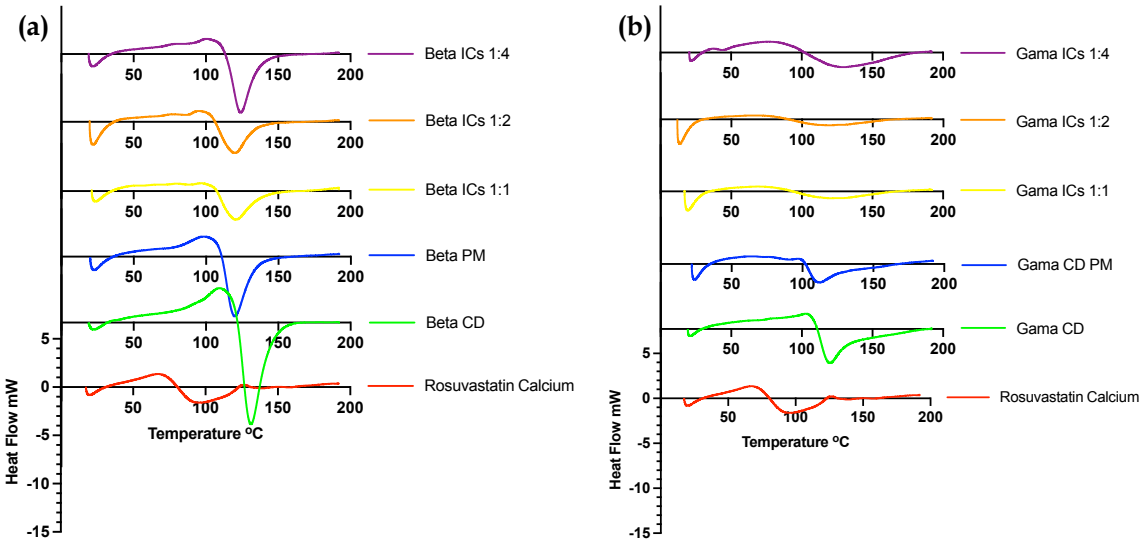

Figure 3. DSC thermograms of (a) RSV, β CD, physical mixture and inclusion complexs of β CD and RSV (b) RSV, γ CD, physical mixture and inclusion complexs of γ CD and RSV.

Taken together of RSV β CD and γ CD complexs thermograms (Fig. 3), one endothermic peak was observed which was assigned to water loss of βCD and γ CD. This endothermic peak shifted and intensity is also strongly reduced in all complexes due to interactions between RSV and CDs during complexation except for at 1:4 ratio of γ and β CD ICs. In particular, the thermogram of 1:4 RSV-βCD ICs was also quite similar to the physical mixing thermogram. This result was considered the presence of free drug and cyclodextrin as a result of not forming a complete inclusion complex at 1:4 ratio of β CD ICs. Besides that, the absence of endothermic peaks of RSV was observed in all ICs thermograms indicating successful and complete complexation of RSV with βCD and gamma CDs [21].

2.2.2. Fourier Transform Infrared Spectroscopy (FT-IR) analysis

Possible interaction between the functional groups and structures of the drug and the cyclodextrins as a result of the formation of the inclusion complex was evaluated by FT-IR analysis. The overlay of the obtained IR spectra of pure RSV, β CD and γ CD, along with their physical mixtures and all inclusion complexes, are presented in Figure 4. The IR spectrum of RSV displayed certain characteristic peaks of aromatic N-H stretching (3337 cm−1), C–H stretching (2968, 2931 and 2872 cm−1), C=O stretching (1435 cm−1) and C–N bond stretching (1600-1378cm−1) like those reported in the literature [2, 6]. Due to similar chemical structures of pure β and γ CD, basically same peaks intensity and wavelengths have emerged. Both cyclodextrin spectra revealed intense absorption band of free O–H group at around 3200-3400 cm−1, C–H stretching vibrations in range of 2930-2920 cm−1, short absorption band of hydrated (H–O–H) group appeared in the 1650–1640 cm−1 region and C–O–C bending and C-O stretching band observed at

Journal of Research in Pharmacy
Research Article

approximately 1020, 1076 and 1150 cm−1. These were almost same as reported in the literature [20, 24]. Complex formation in FT-IR analyzes is usually indicated by the disappearance or broadening or the change in intensity and wavelengths of the characteristic peaks [25]. In the physical mixtures obtained with both beta and gamma CD, peaks similar to the characteristic peaks of RSV and CDs are located in the spectras. It revealed that no chemical interaction and destabilization had occurred between the cyclodextrins and the drug. However, when inclusion complexations are compared with physical mixtures, remarkable differences are observed in characteristic peaks of individual components. In the spectrums of all γ CD ICs, a single peak appeared in the C-H stretching band region of cyclodextrins at around 2920 cm−1 whereas olefinic C-H vibrations observed at 2968, 2931 and 2872 cm−1 wavelengths of RSV vanished. On the other hand, in case of β CD ICs, major peak broadening and shifting were distinguished in C-H vibrations band. In addition, after the formation of ICs with both CDs, the absorption peaks of carbonyl C=O stretching and C–N bond stretching at 1435 cm−1 and 1600-1378cm−1 were slightly shifted with a marked decrease in intensity. It has been reported that the utilization of hydrogen atoms for intermolecular bond formation causes a reduction in the frequency of the characteristic peak in complexation [26]. Therefore, these differences were considered that existence of hydrogen bond interactions between the carbonyl and amine groups of RSV and the hydroxyl groups of γ CD. Bottom line, carbonyl and amine groups were involved in integrating drug molecules into the CD cavity [27]. Moreover, for both spectras of β CD ICs and γ CD ICs, increased intensity was observed in the broad band at around 3,300-3,400 cm-1, compared to the characteristic peaks of the main components (O-H strectching of CDs and cyclic amine N-H strectching of RSV). This result was attributed to the hydrogen bonds occured by the complexation [26]. Complexation with γ C and β CD has completely modified spectral regions of individual components suggesting stable inclusion complex formation.

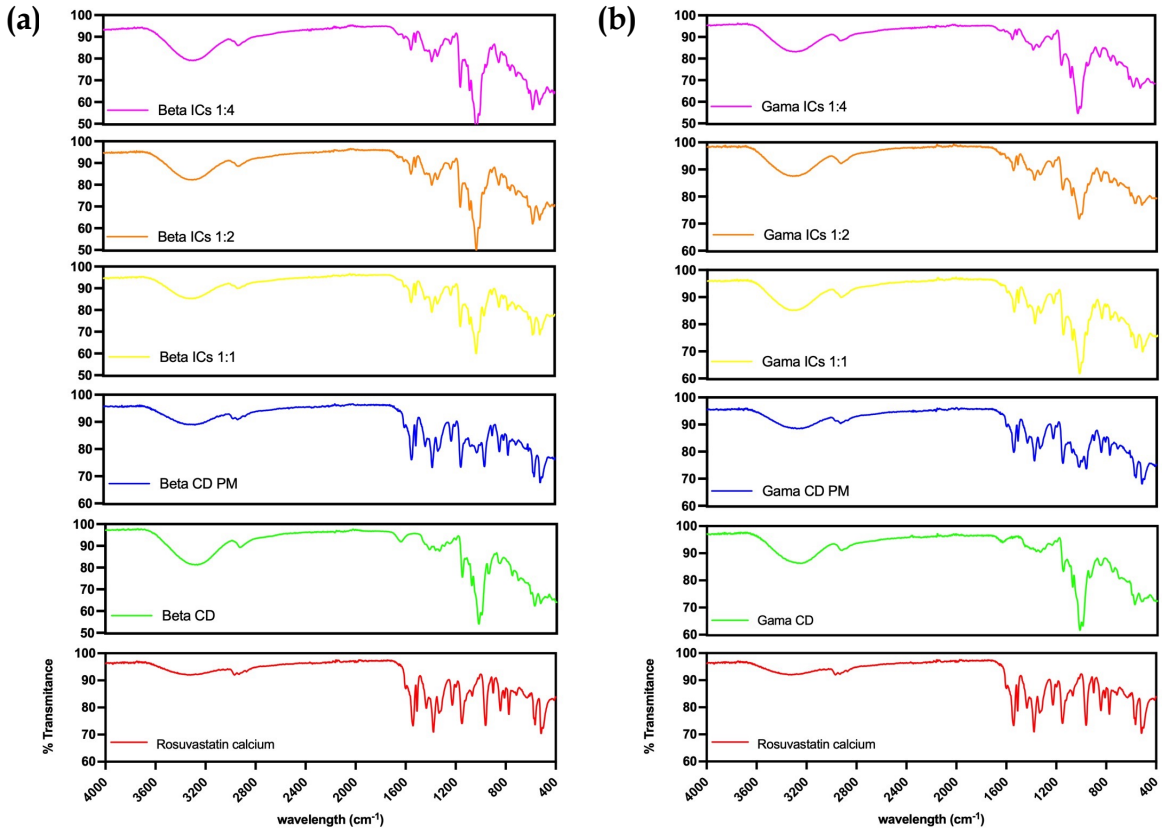

Figure 4. FT-IR spectrums of (a) RSV, β CD and their physical mixtures and inclusion complexes, (b) RSV, γ CD and their physical mixtures and inclusion complexes.

http://dx.doi.org/10.29228/jrp.740
J Res Pharm 2024; 28(3): 797-807

Journal of Research in Pharmacy
Research Article

2.3. Solubility and Drug Loading Efficiency Studies

The solubility of RSV in water increased respectively from 7.76 mg/mL to 10.47, 10.63 and 9.96 mg/mL for β CD ICs, and 11.23, 13.31 and 15.54 mg/mL for γ CDs at 1:1, 1:2 and 1:4 molar ratio. As anticipated, RSV solubility was enhanced after complexation with both cyclodextrin derivatives. The increase in the solubility could be observed as a result of the wetting property and hydrophilicity of the CD as well as the incorporation of the drug into the hydrophobic cavity of the CD [28]. Therefore this result confirms the formation of stable inclusion complexes between the RSV and both CDs alongside with obtained Ks values. In addition, dissimilar enhancements in water solubility were observed between cyclodextrins and molar ratios. The calculated fold increases in water solubility of RSV with β CD ICs and γ CD ICs were also illustrated in Figure 5. The fold increase results showed that ICs with β CD have increased solubility of RSV about 1.3 times regardless of CD molar ratios ($p>0.05$). As compared to ICs with β CD, the solubility of RSV was significantly greater in ICs with γ CD and increased up to 1.45, 1.72 and 2.0 fold at 1:1, 1:2 and 1:4 molar ratio, respectively. Conspicuously, RSV solubility increased in relation to the molar ratio of γ CD ($p<0.05$). The observed solubility increase in line with the γ CD molar ratio could be attributed as the formations of noninclusion complexes and complex aggregates that capable of dissolving drug with micelle-like structures, beside drug-cyclodextrin inclusion complex formation [17]. As a result, while both CDs ICs significantly provided an increase in solubility compared to pure RSV, in comparison ($p<0.05$), γ CD ICs achieved higher effect on solubility enhancement than β CD ICs suggesting γ CD is a more efficient solubilizer for RSV ($p<0.05$). This finding may be due to the lower Ks value of β-CDs obtained as a result of the less affinity of β-CD to RSV [29]. Additionally, 1:4 was considered to be optimum ratio in inclusion complexes for solubility enhancement since the highest solubility is observed at 1:4 molar ratio for γ CD ICs.

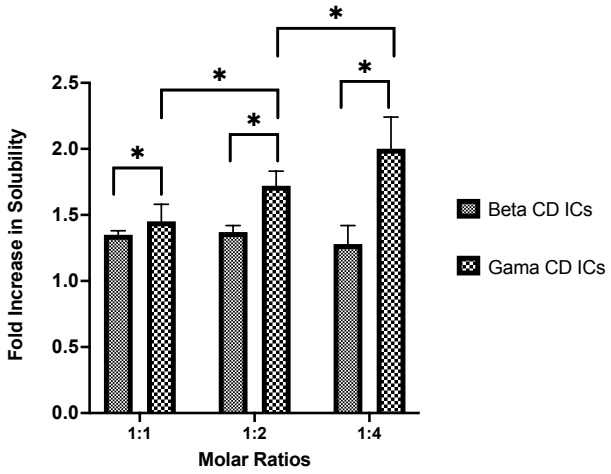

Figure 5. Fold increase in water solubility of RSV with β CD ICs and γ CD ICs (mean ± SD, n = 3), * $p < 0.05$.

The drug loading efficiency % values were found 99.8 ± 0.81 (at 1:1), 99.4 ± 2.49 (at 1:2), 99.9 ± 1.33 (at 1:4) for βCD ICs and 100.7 ± 1.67 (at 1:1), 101.8 ± 0.91 (at 1:2), 100.6 ± 2.23 (at 1:4) for γ CD ICs. As a result, high drug loading efficiency with more than 99.41 % RSV entrapment was achieved for all ICs.

2.4. In-Vitro Dissolution Study

The dissolution profile of the pure RSV and ICs examined at pH 6.6 citrate buffer at 50 rpm for 1 hour according to RSV tablet USP monograph. The percantage cummulative dissolved RSV from pure RSV, β CD ICs and γ CD ICs were presented in Fig. 6. Due to the amorphous solid form of RSV as evidenced by the DSC thermogram, the dissolution rate of pure RSV was observed relatively lower than ICs. The Pure RSV dissolution rate increased from 59.68% to 91.76% in the one hour period from the first time point. Compared with pure RSV, all the prepared ICs enhanced the dissolution rates irrespective of CD type and molar ratio. Especially in the first release minutes, dissolution rate is obviously faster for the all ICs. Thus all ICs demonstrated an improved immediate release profile due to the complexation with hydrogen bonds between drug and CDs that increases solubility of the drug and leads to rapid drug release by reducing the particle size to the molecular level and increasing the surface area. [30, 31]. Therefore, the enhancement in the dissolution rate indicates a successful complex formation between the drug and CD. In case of multiple

point comparison of dissolution profile of both γ and β ICs, γ CD ICs showed higher drug release than β CD ICs. Insomuch that, at the molar ratios of 1:2 and 1:4, RSV release was almost completed in the 5th minute. Moreover, just as the solubility improved in relation to the γ CD molar ratio in the solubility study, RSV dissolution rates also reasonably increased with γ CD molar ratio. Particularly γ CD ICs at 1:4 ratio released the highest RSV with 95.12 % at 3 min and 100% completion in 15 min whereas pure RSV dissolved 59.68% at 3 min. Observing γ CD molar ratio-dependent increase in both solubility and dissolution rate results supported the idea of noninclusion complexes and complex aggregates formations for γ CD ICs.

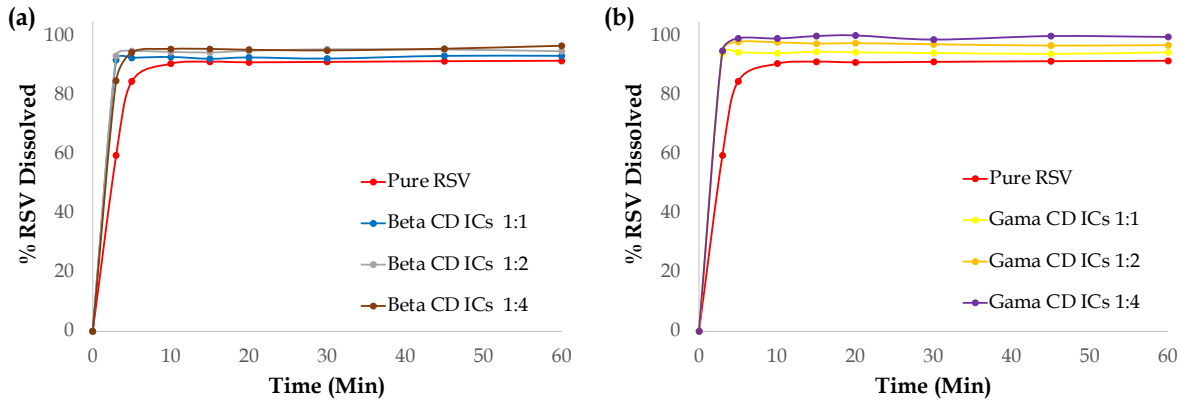

Figure 6. Dissolution profiles of (a) pure RSV and β CD ICs (b) RSV and γ CD ICs at pH 6.6 citrate buffer (mean ± SD, n = 3)

Calculated dissolution efficiencies at 10 min (DE10), 30 min (DE30) and 60 min (DE60) of RSV and all ICs were exhibited in Figure 7. At 10 min time points, all ICs revealed a significant increase in DE over pure RSV ($p<0.05$), and this increase persisted at 30 minutes except for Beta 1:2 ICs. Since high solubility values were attained at the 60th minute, no difference was observed between RSV and ICs DE values at this time point ($p>0.05$). Comparing the dissolution efficiency data turned out once more that enhanged dissolution rate with ICs was especially prominent at the beginning of dissolution. In general, β CD ICs demonstrated a slight lower improvement in dissolution efficiency than γ CD ICs. Higher dissolution efficiency could be obtained by converting RSV into a more soluble, amorphous and wetting-ability form after inclusion complexation [32]. Thus the more enhancement in the dissolution rates for γ CD ICs could be attributed to the higher RSV solubility results. As an outcome, the dissolution capacity of β CD was found to be lower for a poorly aqueous soluble drug, RSV as previously reported in a different poorly aqueous soluble drug by Tang et al. [33].

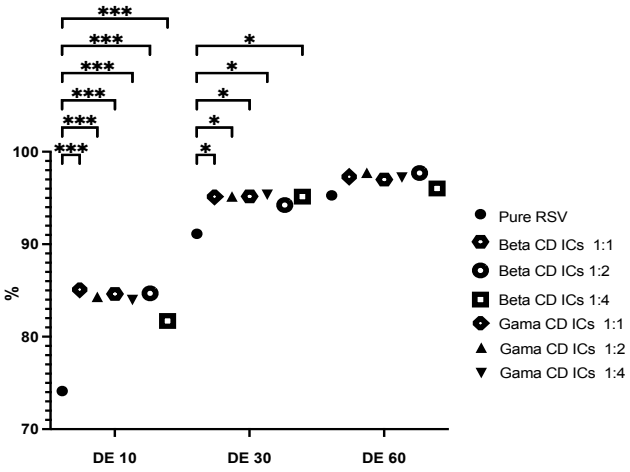

Figure 7. Dissolution efficiencies of RSV, β CD ICs and γ CD ICs at 10 min (DE10), 30 min (DE30) and 60 min (DE60), * $p<0.05$, *** $p<0.001$ as compared to RSV.

3. CONCLUSION

In this present work, β and γ cyclodextrin complexes were evaluated with different molar ratio by using freeze drying method in order to improve solubility and dissolution rate of a BCS Class II drug RSV. Considering the outcomes discussed above, it was observed that β CD and γ CD formed inclusion complexes with RSV for all drug:CD molar ratios. The FT-IR and DSC results demonstrated the host-guest interaction between RSV and CDs. In addition to this, the increased RSV solubility and Ks values of ICs suggested the successful complexation of RSV with β CD and γ CD. The solubility and dissolution results revealed that ICs of both CD derivatives gave a significant increase in RSV solubility and dissolution rate, also caused an immediate release profile. In particular, γ CD ICs displayed higher improved complexation and solubility as well as dissolution rate than β CD ICs. Also, in line with the increasing γ CD molar ratio, a better increase on solubility and dissolution rate were provided. Therefore, it can be concluded that γ CD complexation of RSV is more appropriate for improving the RSV solubility and consequently its bioavailability.

4. MATERIALS AND METHODS

4.1. Materials

Rosuvastatin calcium was given as a kind gift by ILKO Pharmaceuticals to be used in our research studies. β CD and γ CD (β CD and γ CD) were purchased from from Wacker Chemie AG as pharmaceutical grade products (Cavamax® W7 Pharma and Cavamax ® W8 Pharma). All other reagents were of analytical grade and purified water was used throughout the study.

4.2. Phase Solubility Studies

Phase solubility studies were carried out in aqueous medium at room temperature in line with the method reported by Higuchi and Connors [18]. Briefly, excess amount of RSV was added to 2 ml aqueous solution containing increasing concentrations of β CD or γ CD 0, 1, 2, 4, 6, 8, 10 mM. Three parallel measurements were performed at all CD concentration points. In order to reach equilibrium, obtained suspensions were stirred on a horizontal shaker at 300 rpm for 48 hours at room temperature. After 48 h, the suspensions were sequentially centrifuged at 10,000 rpm for 15 minutes, then the supernatants were filtered through 0.45 µm PTFE syringe filter. Appropriately diluted RSV concentrations were measured spectrophotometrically in a Shimadzu UV-Vis spectrophotometer (UV-1800) at 240 nm. The phase solubility diagram of RSV in aqueous solution was generated by plotting the measured RSV concentration (mM) against the CD concentration (mM). The apparent stability constants, Ks were calculated from the phase solubility diagram by using Equation (1) assuming 1:1 complex formation at each point. The slope values in the equation were obtained from the initial linear line of the phase solubility diagram and experimentally determined the intrinsic solubility of RSV in an aqueous solution without CDs was represented as S_0.

$$Ks=slope/[S_0(1-slope)] \quad \text{(Eq.1)}$$

4.3. Preparation of Inclusion Complexes and Physical Mixture

ICs of RSV were prepared with β CD and γ CD by the freeze-drying method at different RSV:CD molar ratios of 1:1, 1:2 and 1:4. Inclusion complexations were named according to the CD type and molar ratio of RSV:CD, as Gama 1:1, Gama 1:2 and Gama 1:4 for γ CD ICs; as Beta 1:1, Beta 1:2 and Beta 1:4 for β CD ICs. Firstly β CD or γ CD was dissolved in 10 mL purified water at specified molar ratio and room temperature. Afterwards the stoichiometric amounts of RSV CD was added to the CD solution and stirred on a magnetic stirrer at 500 rpm for 24h. The obtained solutions were filtered with a 0.45 µm PTFE syringe filter and then frozen at −20 °C overnight. The completely frozen aqueous solution were freeze dried at −80 °C for 48 h using a lyophilizer (Martin Christ Freeze-dryer Gamma 1-16 LSCplus, Germany). The resulting solid ICs were stored in an airtight colored bottle at +4-8°C in a refrigerator until use. The physical mixtures (PM) were freshly prepared by mixing RSV and CDs weighed in corresponding stoichiometric amounts (1:1 molar ratio) in a ceramic mortar [27].

4.4. Physicochemical Characterization

ICs was characterized physicochemically by fourier transform infrared spectroscopy and Differential Scanning Calorimetry to ensure inclusion complexation and stability of the complex between RSV and β CD and γ CD. DSC thermograms and FT-IR spectrums of rosuvastatin, β and γ CDs alone as well as all ICs and physical mixtures were taken separately.

4.4.1. Differential Scanning Calorimetry (DSC) Analysis

Thermal analysis is performed with the a differential scanning calorimeter (Setaram DSC 131). Samples weighing approximately 5 mg were placed in hermetically sealed aluminum crucibles. Thermograms are obtained in the temperature range of 20°C to 200°C by subjecting the samples to a constant heating rate (10 °C/min) under dynamic nitrogen gas.

4.4.2. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

Infrared spectra of solid. samples were obtained between 4,000–400 cm^{-1} by using IR spectrophotometer (Vertex 70, Bruker, OPUS software) equipped with attenuated transmittance reflectance (ATR). All measurements were performed at room temperature.

4.5. Solubilization Study

In order to analyze the improvement in solubility of β CD and γ CD with different molar ratios, solubility studies were performed in purified water at room temperature [33]. Excess quantities of RSV and β CD ICs and γ CD ICs were added to 2 ml purified water in eppendorf tube. All ICs and RSV were prepared in triplicate. For achieving supersaturated solutions, the suspensions were stirred at 300 rpm for 48 hours at room temperature on a horizontal shaker. Subsequently, the same procedures as in the phase resolution study were applied. To show the difference in solubility increase clearly, the fold increase values in RSV solubility of β CD ICs and γ CD ICs were calculated following Equation (2);

Fold increase in solubility = RSV solubility in ICs / Pure RSV solubility (Eq.2)

4.6. Drug Loading Efficiency

To determine drug loading efficacy of β CD ICs and γ CD ICs, all inclusion complex equivalent to 10 mg of RSV were weighed and dissolved in 10 mL methanol. The mixture was stirred for 2 h on a horizontal shaker at room temperature. After that, the solution were filtered through 0.45 µm PTFE syringe filter. Obtained solution suitably diluted and spectrophotometricly analysed following the same procedure performed for the phase solubility studies. For all ICs, experiments were conducted three times. Drug loading efficacy was calculated from the measured RSV concentrations using the following Equation (3).

Drug Loading Efficacy (%)=(Experimental RSV Content)/(Initial RSV Content)x100 (Eq.3)

4.7. In-Vitro Dissolution Study

Dissolution studies of pure RSV and ICs were conducted according to RSV tablet USP monograph by using the paddle method (Apparatus 2) on dissolution tester, Pharma test PTWS 120D (Hainburg, Germany). The dissolution medium comprised of 900 ml of pH 6.6 citrate buffer at 37.0 ± 0.5°C with a stirring speed of 50 rpm. 10 mg RSV and equivalent amount of ICs to 10 mg of RSV were separately added in three parallel dissolution vessel. At predeterminated time intervals (3, 5, 10, 15, 20, 30, 45, and 60 min), 5 mL samples were withdrawn and replaced with equal volume of dissolution medium. All samples were filtered through 0.45 µm PTFE syringe filter and then RSV concentrations of the filtrates were analysed with same spectrophotometric procedure applied for the phase solubility studies. The trapezoidal method was used to calculate the dissolution efficiency (DE) using Equation (4).

$$DE = \frac{\int_0^t y.dt}{y100.t} .100\%$$

(Eq.4)

where y is the percentage of dissolved drug at time t, and y100.t is the area of the rectangle corresponding to a complete dissolution at time t.

4.8. Statistical Analysis

Statistical analysis was performed via Mann Whitney U test for comparisons between independent groups. A p value of 0.05 or less was considered as statistically significant. All statistical analyses were conducted using GraphPad Prism 6.01 (GraphPad, Inc., San Diego, CA, USA).

This is an open access article which is publicly available on our journal's website under Institutional Repository at http://dspace.marmara.edu.tr.

Acknowledgements: This study was not supported financially.

Author contributions: Concept – D.O.; Design – D.O.; Supervision – D.O.; Resources – D.O.; Materials – D.O.; Data Collection and/or Processing – D.O.; Analysis and/or Interpretation – D.O; Literature Search – D.O.; Writing – D.O.; Critical Reviews – D.O.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications Int J Pharm. 2011;420(1):1-10. https://doi.org/10.1016/j.ijpharm.2011.08.032

[2] Sarfraz RM, Ahmad M, Mahmood A, Minhas MU, Yaqoob A. Development and evaluation of rosuvastatin calcium based microparticles for solubility enhancement: an in vitro study. Adv Polym Technol. 2017;36(4):433-441. https://doi.org/10.2147/DDDT.S143712

[3] Rodriguez-Aller M, Guillarme D, Veuthey J-L, Gurny R. Strategies for formulating and delivering poorly water-soluble drugs. J Drug Deliv Sci Technol. 2015;30:342-351. https://doi.org/10.1016/j.jddst.2015.05.009

[4] Quirk J, Thornton M, Kirkpatrick P. Rosuvastatin calcium. Nat Rev Drug Discov. 2003;2(10):769-770. https://doi.org/10.1038/nrd1205

[5] Rosenson RS. Rosuvastatin: a new inhibitor of HMG-coA reductase for the treatment of dyslipidemia. Expert Rev Cardiovasc Ther. 2003;1(4):495-505. https://doi.org/10.1586/14779072.1.4.495

[6] Gonzalez R, Pena MA, Torres NS, Torrado G. Design, development, and characterization of amorphous rosuvastatin calcium tablets. PLoS One. 2022;17(3):e0265263. https://doi.org/10.1371/journal.pone.0265263

[7] Elsayed I, El-Dahmy RM, Elshafeey AH, Abd El Gawad NA, El Gazayerly ON. Tripling the bioavailability of rosuvastatin calcium through development and optimization of an in-situ forming nanovesicular system. Pharmaceutics. 2019;11(6):275. https://doi.org/10.3390/pharmaceutics11060275

[8] Kanikkannan N. Technologies to improve the solubility, dissolution and bioavailability of poorly soluble drugs. J Anal Pharm Res. 2018;7(1):00198. https://doi.org/10.15406/japlr.2018.07.00198

[9] Rumondor AC, Dhareshwar SS, Kesisoglou F. Amorphous solid dispersions or prodrugs: complementary strategies to increase drug absorption. J Pharm Sci. 2016;105(9):2498-2508. https://doi.org/10.1016/j.xphs.2015.11.004

[10] Serajuddin AT. Salt formation to improve drug solubility. Adv Drug Deliv Rev. 2007;59(7):603-616. https://doi.org/10.1016/j.addr.2007.05.010

[11] Elder DP, Holm R, De Diego HL. Use of pharmaceutical salts and cocrystals to address the issue of poor solubility. Int J Pharm. 2013;453(1):88-100. https://doi.org/10.1016/j.ijpharm.2012.11.028

[12] Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Deliv Rev. 2007;59(7):645-666. https://doi.org/10.1016/j.addr.2007.05.012

[13] Carneiro SB, Costa Duarte FÍ, Heimfarth L, Siqueira Quintans JS, Quintans-Júnior LJ, Veiga Júnior VFD, Neves de Lima ÁA. Cyclodextrin¯drug ınclusion complexes: In vivo and ın vitro approaches. Int J Mol Sci. 2019;20(3):642.https://doi.org/10.3390/ijms20030642

[14] Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: basic science and product development. J Pharm Pharmacol. 2010;62(11):1607-1621. https://doi.org/10.1111/j.2042-7158.2010.01030.x

[15] Salústio PJ, Pontes P, Conduto C, Sanches I, Carvalho C, Arrais J, Marques HM. Advanced technologies for oral controlled release: cyclodextrins for oral controlled release. AAPS PharmSciTech. 2011;12(4):1276-1292. https://doi.org/10.1208/s12249-011-9690-2

[16] Adeoye O, Cabral-Marques H. Cyclodextrin nanosystems in oral drug delivery: A mini review. Int J Pharm. 2017;531(2):521-531. https://doi.org/10.1016/j.ijpharm.2017.04.050

[17] Jambhekar SS, Breen P. Cyclodextrins in pharmaceutical formulations I: Structure and physicochemical properties, formation of complexes, and types of complex. Drug Discov Today. 2016;21(2):356-362. https://doi.org/10.1016/j.drudis.2015.11.017

[18] Higuchi T. Phase-solubility techniques. Adv Anal Chem Instr. 1965; 4: 117-212

[19] Ikeda H, Fukushige Y, Matsubara T, Inenaga M, Kawahara M, Yukawa M, Fujisawa M, Aki H. Improving water solubility of nateglinide by complexation of β-cyclodextrin. J Therm Anal Calorim. 2016; 123: 1847-1850. https://doi.org/10.1007/s10973-015-4714-x

[20] Sadaquat H, Akhtar M. Comparative effects of β-cyclodextrin, HP-β-cyclodextrin and SBE 7-β-cyclodextrin on the solubility and dissolution of docetaxel via inclusion complexation. J Incl Phenom Macrocycl Chem. 2020; 96: 333-351. https://doi.org/10.1007/s10847-020-00977-0

[21] Shukla SK, Chan A, Parvathaneni V, Kanabar DD, Patel K, Ayehunie S, Muth A, Gupta V. Enhanced solubility, stability, permeation and anti-cancer efficacy of Celastrol-β-cyclodextrin inclusion complex. J Mol Liq. 2020; 318: 113936. https://doi.org/10.1016/j.molliq.2020.113936

[22] Al-Shdefat R, Anwer MK, Fayed MH, Alsulays BB, Tawfeek HM, Abdel-Rahman RF, Soliman GA. Preparation and evaluation of spray dried rosuvastatin calcium-PVP microparticles for the improvement of serum lipid profile. J Drug Deliv Sci Technol. 2020;55:101342. https://doi.org/10.1016/j.jddst.2019.101342

[23] Ângelo ML, Ruela ALM, Ferreira ACM, Ramos MVdF, Montanari CM, Silva LMd, Araujo MBd. Evaluating the discriminatory power of a dissolution assay for rosuvastatin calcium capsules: Solid-state properties and dissolution media. Braz J Pharm Sci. 2019;55:e17520. https://doi.org/10.1590/s2175-97902019000117520

[24] Li H, Zhang G, Wang W, Chen C, Jiao L, Wu W. Preparation, characterization, and bioavailability of host-guest ınclusion complex of ginsenoside re with gamma-cyclodextrin. Molecules. 2021; 26(23):7227. https://doi.org/10.3390/molecules26237227

[25] Szabó ZI, Orbán G, Borbás E, Csicsák D, Kádár S, Fiser B, Dobó M, Horváth P, Kiss E, Budai L, Dobos J, Pálla T, Őrfi L, Völgyi G, Tóth G. Inclusion complexation of the anticancer drug pomalidomide with cyclodextrins: fast dissolution and improved solubility. Heliyon. 2021;7(7):e07581**.** https://doi.org/10.1016/j.heliyon.2021.e07581

[26] Vyas A. Preparation, characterization and pharmacodynamic activity of supramolecular and colloidal systems of rosuvastatin–cyclodextrin complexes. J Incl Phenom Macrocycl Chem. 2013;76:37-46. https://doi.org/10.1007/s10847-012-0170-4

[27] Das S, Subuddhi U. Studies on the complexation of diclofenac sodium with β–cyclodextrin: Influence of method of preparation. J Mol Struct. 2015; 1099: 482-489. https://doi.org/10.1016/j.molstruc.2015.07.001

[28] Sapte S, Pore Y. Inclusion complexes of cefuroxime axetil with β-cyclodextrin: Physicochemical characterization, molecular modeling and effect of l-arginine on complexation. J Pharm Anal. 2016;6(5):300-306. https://doi.org/10.1016/j.jpha.2016.03.004

[29] Aleem O, Kuchekar B, Pore Y, Late S. Effect of beta-cyclodextrin and hydroxypropyl beta-cyclodextrin complexation on physicochemical properties and antimicrobial activity of cefdinir. J Pharm Biomed Anal. 2008;47(3):535-540. https://doi.org/10.1016/j.jpba.2008.02.006

[30] Ridhurkar DN, Ansari KA, Kumar D, Kaul NS, Krishnamurthy T, Dhawan S, Pillai R. Inclusion complex of aprepitant with cyclodextrin: evaluation of physico-chemical and pharmacokinetic properties. Drug Dev Ind Pharm. 2013;39(11):1783-1792.https://doi.org/10.3109/03639045.2012.737331

[31] Devasari N, Dora CP, Singh C, Paidi SR, Kumar V, Sobhia ME, et al. Inclusion complex of erlotinib with sulfobutyl ether-β-cyclodextrin: Preparation, characterization, in silico, in vitro and in vivo evaluation. Carbohydr. Polym. 2015;134:547-556. https://doi.org/10.1016/j.carbpol.2015.08.012

[32] Maqbool I, Akhtar M, Ahmad R, Sadaquat H, Noreen S, Batool A, Khan SU. Novel multiparticulate pH triggered delayed release chronotherapeutic drug delivery of celecoxib-β-cyclodextrin inclusion complexes by using Box-Behnken design. Eur J Pharm Sci. 2020;146:105254. https://doi.org/10.1016/j.ejps.2020.105254

[33] Tang P, Li S, Wang L, Yang H, Yan J, Li H. Inclusion complexes of chlorzoxazone with β-and hydroxypropyl-β-cyclodextrin: characterization, dissolution, and cytotoxicity. Carbohydr Polym. 2015; 131: 297-305. https://doi.org/10.1016/j.carbpol.2015.05.055