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ORIGINAL ARTICLE

Characterization of silk fibroin based films loaded with rutin–β-cyclodextrin inclusion complexes

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Abstract In this study, cyclodextrin inclusion complexes with rutin were prepared via co-precipitation method. Stability constant and solubility energy of beta-cyclodextrin complex were calculated as 262 M^{-1} and 1,737 kJ mol^{-1}, respectively. Aqueous solubility of rutin was increased with inclusion complex of beta-cyclodextrin. The effect of temperature on both aqueous solubility of free rutin, and its inclusion complex was also studied. Characterization of cyclodextrin complexes were conducted with UV–Vis spectrophotometry, Fourier transform infrared spectroscopy, X-ray diffractometry, differential scanning calorimetry, thermal gravimetric analysis, nuclear magnetic resonance spectroscopy and scanning electron microscopy techniques. Characterization results supported formation of inclusion complexes. Dissolution profiles of rutin, physical mixture and inclusion complex of rutin were observed at 37 °C. Dissolution results proved the effect of cyclodextrin addition on solubility rate of rutin. After loading rutin and its complexes into silk fibroin based films, release tests were performed at 37 °C in neutral pH conditions for 24 h. Most of the rutin were released from silk fibroin films within the first 5 h and the rest of it was released slowly (sustained release). Electron microscope analyses showed that films had homogenous and dense morphologies. These results revealed that silk fibroin is useful for preparing bioactive films loaded with natural compounds and for modifying their release behaviour at physiological conditions.

Keywords Rutin · Beta-cyclodextrin · Inclusion complex · Silk fibroin Film

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Introduction

Host–guest complexes of cyclodextrins (CDs) with natural compounds have been studied extensively via researchers from different disciplines [1–5]. Rutin (Rt) is a naturally occurring phenolic flavonol glycoside, which is named as vitamin P and has been to be an activating factor for vitamin C in the nutrition literature [6].

Rutin (2-3,4-dihydroxyphenyl-3,5,7-trihydroxy-4-oxo-chromen-3yl rutinoside) is an *o*-glycoside composed of aglycon of quercetin and rutinose. Rt present in many of the plants. Beside the beneficial effects of Rt, its low aqueous solubility causes this substance to have lower in vivo antioxidant and/or anticarcinogenic activities compared with its in vitro activities. Also, this compound is very sensitive to temperature, light, oxygen; it can easily degrade via these factors [7]. At this point utilization of CDs would provide a practical solution to these problems. CDs are cyclic molecules composed of glucopyranose ring units to form truncated cone type, doughnut structures. The exterior of the CDs are hydrophilic while the interior is hydrophobic, and the different CDs possess different cavity sizes according to the number of glucopyranose rings present [8]. This structure provides CDs to form complex compounds with the molecules having ability to locate the cavity. The most common are the α, β, and γ-CDs (alpha-, beta- and gama-cyclodextrins) which are composed of six, seven, and eight sugar units respectively [9]. Inclusion

complexes have been used in food and drug industry for many years; for example, they have been used in order to control the activity and/or dose of flavonoids in the body [7]. As a general consideration in most of the researches β-CD has been preferred as the host molecule mainly because of availability and practical reasons. Also, some specific properties of β-CD (i.e. water solubility, pseudo polymorphism, etc.) is more preferable when compared to other CDs [10]. The host cavity size is critical for the drug's locating ability into CD [11]. The interactions responsible for the association of guest molecules with β-CD are mainly van der Waals interactions, hydrogen bonds and hydrophobic interactions [12]. For obtaining complexes that have higher shelf life, β-CD is preferred because of its optimal internal cavity size, so that the 'best fit' arrangement between guest and host could be achieved [13].

The stability constant and stoichiometry of the inclusion complexes, depending on the guest/host concentration employed are useful indices for estimating the binding strength of the complex and changes in the physicochemical properties of the guest molecule in the complex. Moreover, attention should be directed towards various environmental factors, such as dilution, temperature, pH, and additives, in the design of practical formulations and routes of administration [14]. In the literature, highest stability constants were obtained with β-CD and its hydrophilic derivative hydroxypropyl-β-CD [15, 16]. Longer time and more harsh conditions needed to degrade complexes that have higher stability constant values [14].

The physicochemical properties of guest molecules can easily be modified as a result of the interactions between guest molecules and CD's host cavity [17]. CDs have been found to be safe enough to be used in food and drug systems. Therefore, cyclodextrins and their derivatives have found a place in a number of consumer products and in many industrial processes [8]. Usage of CD in drug formulations enhance solubility [18]. Complete complex formation can be confirmed by the good dissolution and in vitro diffusion ability of the products hold within the cavity [18].

CDs can be used as a protective layer for sensitive compounds like flavonoids, but sometimes an additional carrier material is needed to modify release properties of flavonoids in advance. Silk fibroin (SF) has been selected as water insoluble carrier material in many studies, since it is a biodegradable and biocompatible biopolymer [7]. Fibroin is a fibrous silk protein, which is produced by silkworm *Bombyx mori*. SF has capability to form pure or blended films with different polymer solutions in aqueous or formic acid based solutions [19].

The aim of this paper is to produce the inclusion complex of Rt with β-CD and to investigate whether compounds can effectively be loaded into SF films. The release behavior of Rt from SF films was also evaluated. There are a few study in literature, that are interested in loading CD complexes into films/membranes [20–22] and there is no study on usage of SF films in literature and this point makes our study unique.

Experimental

Materials

Dialysis tubes were obtained from Milipore Chem. Co. (St. Louis, MO, USA). Formic acid (98–100 %) supplied from Riedel–de Haen. Hydrochloric acid and sodium hydroxide were obtained from Riedel. Phosphate buffered saline (PBS) tablets were purchased from OXOID (Dulbecco's PBS tablets). Rutin (98.5 + %; MW: 664.58) was obtained from Merck Co. (Darmstadt, Germany). β-CD (MW: 1135) obtained from Sigma Aldrich. Silk fibroin was purchased from KOZA BIRLIK (Bursa, Turkey).

Preparation of materials

The inclusion complexes of Rt were prepared with a similar procedure reported in the literature [1, 2, 16, 23]. The solid Rt complexes with β-CD in a molar ratio of 1:1 were obtained by suspending adequate amounts of both molecules in 40 ml deionized water. Stirring was carried out for 144 h, under controlled temperature (25 ± 0.01 °C) and then solutions were centrifuged in order to eliminate the uncomplexed Rt which did not react with β-CD. Samples were frozen at −18 °C and then freeze dried at −45 °C yielding the solid complex as a pale yellow powder.

To form a SF film; the sericin was removed from raw silk fibers. Silk fibers free from sericin were dissolved in Ajisewa's solution [24].This solution was then dialyzed to obtain aqueous silk fibroin solution. To obtain regenerated silk fibroin (RSF), aqueous SF solution was quickly put in a shell freezer and then freeze dried until foam like dry substance was formed. RSF was slowly dissolved in formic acid: water (1:1) binary solvent system. In case of active agent loading, Rt, PM and IC were individually added into film solution and stirred for 2 h. Film solutions were poured into Polystyrene sterile petri dishes and casted under 25 °C within fume hood for 48 h. The dried films were stored in desiccators at 20 °C until they are used in experiments. The solubility of the films was controlled by immersing them in deionised water and buffer solutions.

Measurements

For determination of experimental solubilities; aqueous solutions of Rt between 0 and 0.1 mM concentrations were prepared. The solution was stirred till the Rt was mostly dissolved. The supernatants' absorbances were measured at 255 nm in

Thermo Multiscan UV–Vis spectrophotometer. Absorbance values were used in calculation of experimental solubility values of the samples. Calculation of the solubility of the inclusion complexes were done via spectrophotometric method.

For calculation of dissolution energy; Free Rutin (Rt), inclusion complex (IC) and physical mixture (PM) containing the same amount of rutin were suspended in 50 ml deionized water. These suspensions were placed in water bath at 25, 45, 65 °C and shaked for 6 h. Samples were taken at every one time interval and active constituent concentrations were determined spectrophotometrically. Then, solubility energies were determined with Clasius–Clapeyron equation given below (Eq. 1)

$$\log \frac{c_1}{c_2} = \frac{Q_{sol}}{4.573} * \frac{T_1 - T_2}{T_1 * T_2} \quad (1)$$

where δQ_{sol} dissolution energy (kJ mol^{-1}), T_1, T_2 absolute temperatures (K), c_1 and c_2 solubilities (mM) at $T_1 - T_2$ temperatures.

Phase solubility analyses were also performed by adding excess amount of Rt into β-CD solutions prepared with deionized water at different concentrations (0, 0.01,….,0.30 M). The suspensions were vortexed and sonicated for 1 h and kept on a horizontal rotary shaker (200 rpm) for 3 days. The suspension was filtered in order to obtain a clear solution. All samples were prepared in triplicate. Final clear solutions were diluted properly with deionized water to the calibration curve concentration range, the concentrations of Rt in β-CD solutions were measured with a UV-spectrophotometer at 255 nm. The apparent inclusion rate constant ($K_{1:1}$) was calculated from the slope and intercept of phase solubility curve using the following equation (Eq. 2).

$$K_{1:1} = \frac{slope}{S_0(1 - slope)}, \quad (2)$$

where S_0 is the intrinsic solubility of active compound or drug.

The dissolution profiles of Rt, PM and IC were also evaluated. Rt in excess amount of its aqueous solubility was carefully weighted into erlen mayer flasks, including deionized water and then slowly stirred at 37°C. Aliquots were taken from this solution for every 15 min time intervals and the same volume of fresh medium at the same temperature as that of the tested medium was added immediately. The samples were centrifuged. The concentration of rutin from the supernatant was measured spectrophotometrically at 255 nm with Thermo Multiskan Spectrophotometer. The same procedure was repeated for each sample from PM and IC. All analyses were conducted in triplicate, and the average values were plotted in the graphs.

Characterization

Fourier Transform Infrared Spectroscopy (*FTIR*) analyses were done using the standard KBr pellet technique; samples were scanned in the wavenumber range between 4,000 and 400 cm^{-1} at room temperature under vacuum with Digilab FTS 3000 Mx FTIR spectrophotometer.

By Philips X'pert Pro X-ray diffractometer (XRD)—equipped with Ni filtered CuK_α radiation source- the changes in the crystalline state were monitored over 2θ range of 5°–50°. Measurements with XRD were applied to Rt, β-CD, PM and IC. X-ray diffractograms were used to determine whether crystallinity varied due to different forms of Rt (free, physically mixed or complexed). Relative crystallinity of samples was calculated via area under curve method with ACDLABS 6.0 software. Segal's method were adjusted in order to handle data effectively [25]. See Eq. 3 below.

$$\text{Relative Crystallinity}(\%) = 100 * \left[1 - \frac{(A_{total} - A_{crypeak})}{A_{total}}\right], \quad (3)$$

where $A_{crypeak}$ summation of area of individual crystalline peaks (amorphous region was substracted), A_{total} total area of crystalline and amophous regions.

The variation in thermal stability of Rutin due to its form (free, PM or IC) were also analyzed with Shimadzu TGA-51, thermal gravimetric analyzer (TGA), using stainless-steel pans in the 40–800 °C temperature range at a heating rate of 10°C min^{-1}, under 40 ml min^{-1} nitrogen flow.

The derivative of data were taken and analyzed in order to find the degradation temperatures of samples; derivative thermal gravimetry (dTG or DTG).

Thermal treatment were done for Rt, β-CD, PM and IC, respectively, in the 50–280 °C temperature range at a 10°C min^{-1} heating rate, using aluminium pans under 50.0 ml min^{-1} nitrogen flow with differential scanning calorimetry (DSC) Q10 V9.4 Build 287 (TA Instruments, USA) instrument.

Proton (H-NMR) and carbon nuclear magnetic resonance spectroscopy (C-NMR) analyses were conducted for β-CD, Rt and IC. 5 mg sample was dissolved in Deuterium Dimethyl sulfoxide and analyzed at 21°C with varian NMR spectrophotometer. Fourier transformation of the raw data and the analyses of results were carried out with MestReNova 6.0.2 and ACDLabs 6.0. software.

Scanning electron microscopy (SEM) analyses were performed with Philips XL 30S FEG to detect the morphological structure of the films at 1,000× magnification and the structure of Rt, β-CD, PM and IC at 5,000× magnification. Films were cut into 1 × 1 cm pieces using a

Table 1 Dissolution energy values for free Rt, PM and IC

Form of the compound	t (h)	δQ_{sol} (kJ mol^{-1})		
		25–45 °C	45–65 °C	25–65 °C
Rutin	6	4898.46	7283.97	6016.22
PM	6	4521.56	6573.63	5483.09
IC	6	1737.46	473.15	1145.05

sharp razor for the cross-section observation. The surfaces of samples were coated with gold–palladium (100–200 Å thickness) with sputter coating equipment (Emitech K550X) prior to imaging.

Release of rutin from silk fibroin based films

The release of Rt and IC from SF based films were analyzed using 12 well plates and perforated cartridges within each wells containing release medium (PBS at pH 7.3 or Acetate buffer at pH 4.0), with Thermo Varioskan Spectrophotometer. The well plate was placed in spectrophotometer with horizontal shaking at 100 rpm and thermo stated at 37 °C. At set time intervals, spectroscopic readings were performed for each well plates including blank sample solutions at 255 nm. All experiments were carried out with three samples, and the average values were plotted with standard error bars. The effect of release medium pH [isoelectric point of SF (4.0) and physiological pH (7.3)] on the release behavior of Rt from SF films were investigated at constant temperature (37 °C).

Results and discussion

Solubility and dissolution energy

Initially, aqueous solubility of the free and complexed rutin was determined. Then, the wavelengths, at which maximum absorbances occur (λ_{max}), were determined as 255 and 351 nm for Rt and these values shifted to 260 and 355 nm when it is in the complex form. Maximum solubility of rutin at 25°C was found to be 0.0462 g L^{-1} which was in accordance with the results reported in literature [1, 2, 4, 15, 16, 26]. The maximum solubility of its IC was determined as 15.252 g L^{-1}. Aqueous solubility of rutin at room conditions increased ~330-fold after inclusion complexation.

The changes in dissolution energies with respect to temperature were also observed. These results are tabulated in Table 1. Dissolution energy values has supported the fact that free rutin needs more energy for dissolution as compared to the IC and PM, which proves the occurrence of inclusion complex formation. Results were compatible with the findings of Taneri et al. [27] who used the same method for calculation of solubility energies. As seen in Table 1, at constant temperature range dissolution energies decrease significantly for inclusion complexed form of Rt.

The formation constants for rutin–β-CD inclusion complexes were calculated using phase solubility method with a UV–Vis spectrophotometer. The initial solubility of Rt in water was observed as 0.02 mM at 25 °C. In all of the situations (IC or PM), the results revealed that there was a significant effect ($p < 0.05$) of β-CD addition on solubility value of rutin in its aqueous solution. The solubility of rutin increased linearly with respect to host (β-CD) concentration and the solubility curve was A_L type as reported earlier in the literature [15]. The apparent stability rate constant calculated from the slope and intercept of phase solubility curve, with Eq. 2 was found to be 262 M^{-1} at 25 °C in water. According to stability constant value, we could assume orientation of complexation as 1:1 (1 mol guest: 1 mol host), in accordance with the literature findings (between 260 and 266 M^{-1}) [2, 15, 16]. The correlation coefficient was found to be 0.9987. Phase solubility graph is illustrated in Fig. 1.

Characterization of complexes

Characterization results clearly supported the formation of inclusion complexes. The FTIR spectra of IC and PM were compared with β-CD and Rt, their spectra were presented in Fig. 2. When all of the spectra were analyzed in binary groups as Rt-PM and β-CD–IC; between 400 and 1,700 cm^{-1} wavenumbers, general profile of Rt and PM were very similar, as in the spectra of CD and IC. Data revealed that the optical properties of IC was very similar to that of β-CD, this similarity might prove that Rt was embedded within the β-CD's cavity. At 1654.4 cm^{-1} a carbonyl absorption band due to aromatic ketonic carbonyl stretching was observed for pure Rt molecule. In FTIR spectra of PM compound, a sharp peak was observed at 1,654 cm^{-1} and this peak proved existence of Rt and β-CD separately within the physical mixture. Both of β-CD and IC showed a characteristic secondary cyclic alcohol band at 1,024 and 1,026 cm^{-1} assigned to C–OH stretching vibrations. This band proved Rt was almost fully embedded in CD cavity. In the case of IC in particular, the characteristic aromatic carbonyl-stretching band of Rt shifted to 1,651 cm^{-1}, along with reduced intensity and sharpness of the same band. According to the studies of Sri et al. [16] shifts in the characteristic bands of Rt proved the occurrence of complexation reaction and formation of a new compound.

In the characteristic frequencies of the carboxyl group (–COOH) there were significant changes. This showed that the complex formation altered hydrogen-bonded cyclic

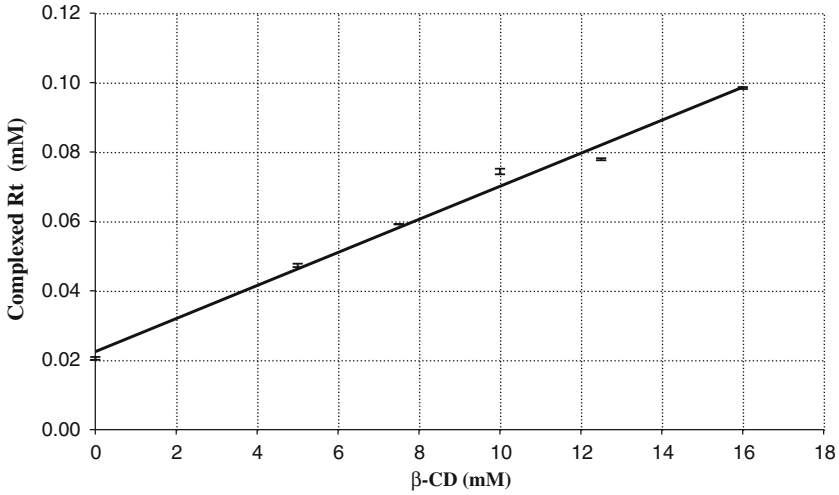

Fig. 1 Phase solubility chart for rutin–β-CD complexes

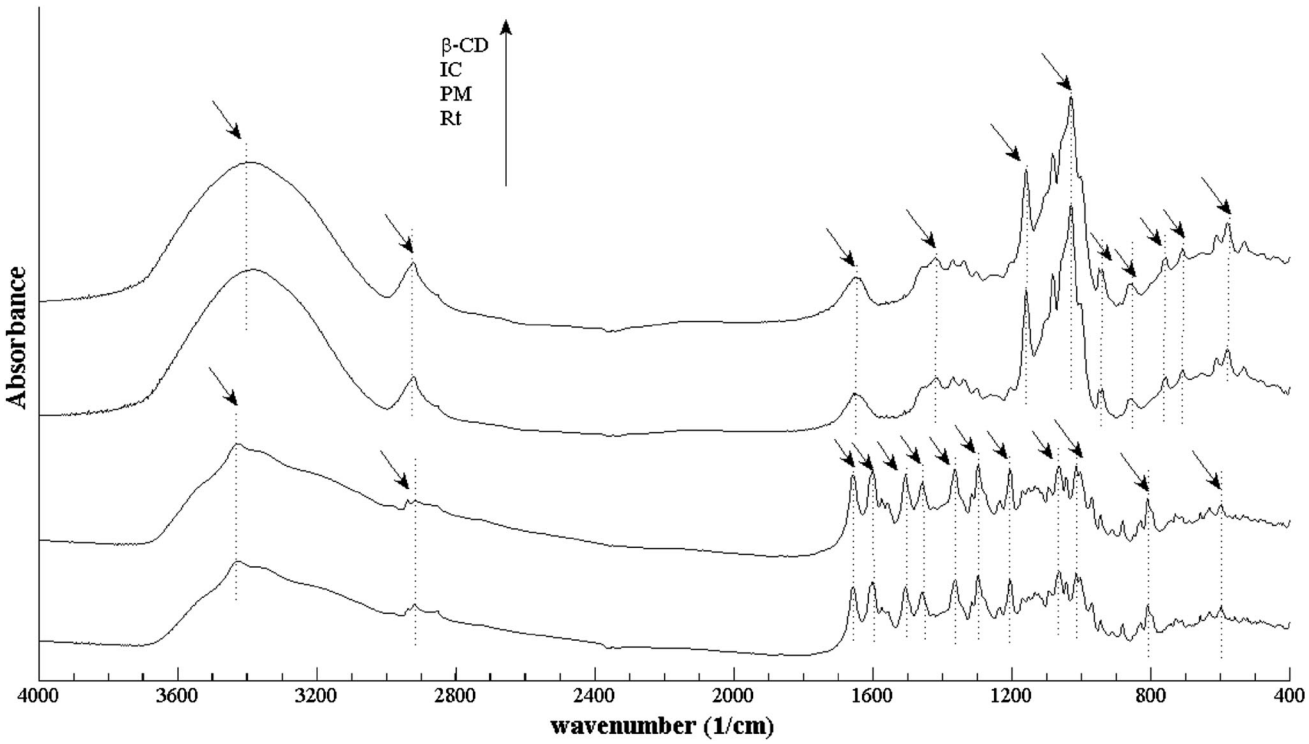

Fig. 2 FTIR spectra of β-CD, IC, PM, Rt

Table 2 FTIR Analysis for Detecting Presence of Complexation Reaction

Type of bond	Wavenumber (cm^{-1})	Shifted to	Vibrational assignments	Reason
C=O	1,709	1,730 cm^{-1}	C=O stretching band (intense)	suggesting a less strong or no H-bonding interaction
C–C–O–H	1,403	1,396 cm^{-1}	C–C–O–H stretching	the strength of the H-bonds decreased and complexation occurred through the carboxyl group
C–O–H	1,271	1,265 cm^{-1}	C–O–H in-plane bending modes	

dimer structure of the carboxyl-group. Characteristic bond types and their wavenumbers that were used in detection of complex formation are tabulated in Table 2.

With XRD analysis it was possible to detect whether inclusion complexation was formed or the compounds were only physically mixed [28]. The samples were analyzed in

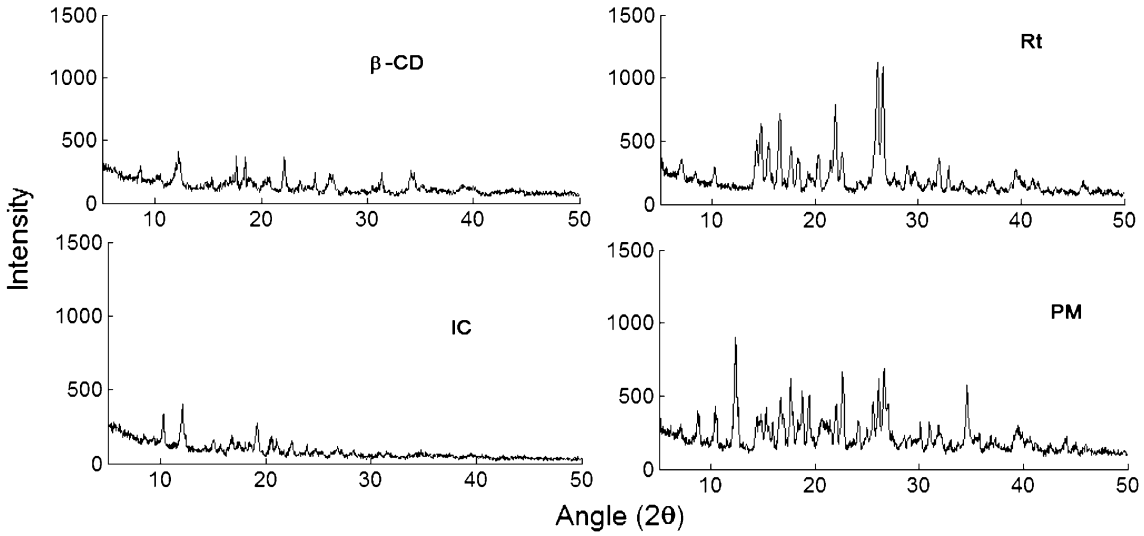

Fig. 3 X-Ray diffractogram of β-CD, Rt, IC and PM

Table 3 Comparison of Relative Crystallinity values of IC, β-CD, Rt, PM

Samples	$A_{crypeak}$	A_{total}	Relative crystallinity (%)
IC	217.9	2293.67	9.50
β-CD	212.98	1678.14	12.69
Rt	2052.16	2902.58	70.70
PM	1686.54	2678.07	62.98

solid state (esp. in finely grinded powder form). The phase of compounds-*crystalline or amorphous*- could be evaluated by comparing peak heights in their diffractograms. The diffraction pattern of the inclusion complex was generally different from pure drug's crystalline form [28, 29]. In literature, results of XRD analyses indicated that the CDs were found in amorphous state [30].

The powder XRD pattern of Rt showed (Fig. 3) highly crystalline nature as evident from the sharp peaks observed at diffraction angle of 14.79°, 16.62°, 22.01°, 26.09° and 26.62° of 2θ values. Crystalline peaks were still detectable in the physical mixture with β-CD at 12.32°, 17.63°, 22.61°, 26.65° and 34.54° of 2θ values.

When we analyze XRD pattern of IC an amorphization was observed. IC was characterized only by large diffraction peaks in which it is no longer possible to distinguish the characteristic peaks of the flavonoids. The peaks for IC were not sharp and had lower intensity when compared with the other corresponding spectra. These results confirmed that Rt was not present as a crystalline material anymore and existed in the amorphous state when complexed with β-CD. Relative crystallinity values of samples were shown in Table 3.

Crystallinity % of β-CD were calculated as 12.69 % which was higher than crystallinity % of IC, that might be evaluated as an interesting result of IC production procedure. IC was dehydrated via lyophilization that cause drying in a short time without any contamination. That's why an additional dehydration step decreased crystallinity ratio. Also existence of crystalline peaks in β-CD's diffractogram could be explained via hygroscopic property of β-CD.

The complex had a new solid phase. XRD results were consistent with thermal analyses results that were obtained with DSC and TGA techniques.

Thermo analytical techniques (TGA, DSC) allowed to detect the real-time changes in mass and heat flow as a function of temperature or time within one sample for each analysis [29]. In thermal techniques it was also possible to observe hypsochromic or bathochromic shifts in phase transition temperatures due to complexation reaction and to make structural/functional identification of the sample before and after decomposition [10].

By DSC analysis the difference between the melting point values of pure and complexed compound can be detected [31].

Thermal analyses were applied to the samples prepared with CD to detect differences between IC and PM and to characterize specific thermal effects, under a controlled heating operation due to molecular entrapment of Rt by CD. Absence of endothermic peaks, which were characteristic for the pure Rt proved the formation of IC as a result of interaction between the Rt and β-CD.

In the first minutes of DSC analysis generally an exothermic shift of the baseline observed that partially dehydrated samples absorbed water (since dehydration is reversible) when exposed to the air moisture in the DSC cell. Generally the first peak of the thermographs of β-CD was proned to the water loss.

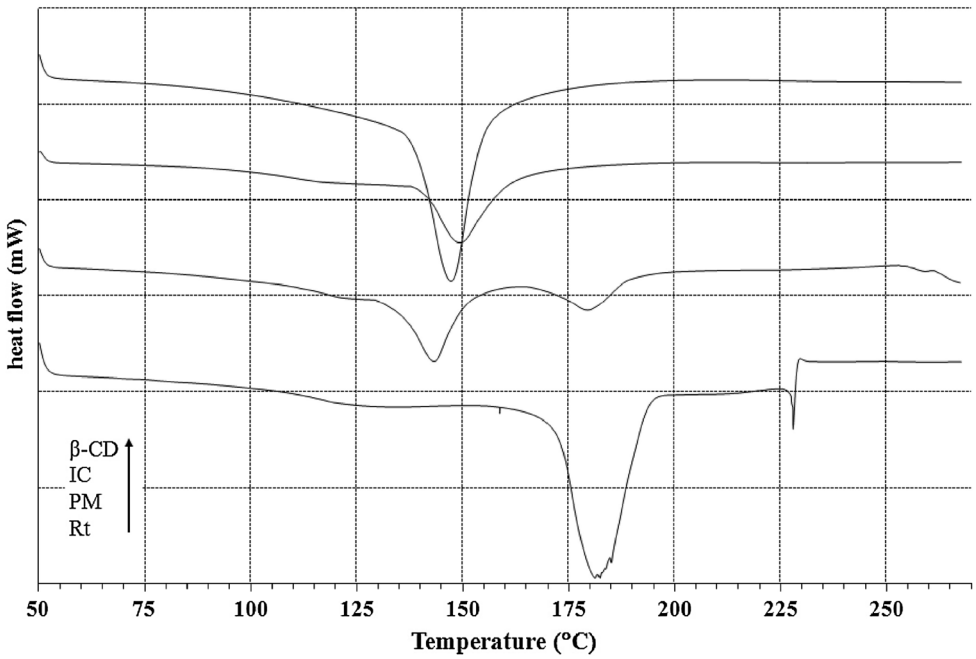

Fig. 4 DSC curves of the compounds; β-CD, IC, PM, Rt

There were several phase transition steps in Rt's calorimetric profile. DSC analyses proved that rutin had a polymorphic structure [32]. The stability of IC was related to the hydrophobicity of the guest molecule. Guest molecule generally tends to relocate with a more hydrophobic molecule. DSC revealed information about phase change temperatures of the compounds. Nature of the included molecule can be contemplated from the shifts in melting temperature values. When guest molecules were embedded in CD cavities or in the crystal lattice, their phase transition temperatures (melting, boiling or sublimation) might disappeared or shifted to a different points (see Fig. 4). β-CD molecule showed a peak at 147 °C and in endothermic, region indicating the melting point at crystalline region. In the DSC diagram of the complex, a shift in β-CD's melting point from 147 to 149 °C was observed. When we compared DSC diagram of Rt molecule with DSC diagram of IC, we have observed some differences within their diagrams. These differences revealed the existence of 'inclusion complex', which showed more similar character to β-CD. The physical mixture of β-CD and Rt gave two peaks at 143 and 180 °C in endothermic region. Two separate peaks might occur due to the existence of two different compounds in sample.

When TGA plot of Rt analyzed in detail, we could observe that the effect of heating rate and environmental atmosphere (N_2, O_2 etc.) on Rt's thermal decomposition behavior was significant. Generally Rt decomposes in four stages between the 30 and 800°C temperature range under nitrogen flow [33]. 30.5 % of Rt decomposes in first three stages and the rest of Rt decomposes at the 4th stage [33]. In thermal analysis of Rt, hydrated forms (dihydrate, trihydrate etc.) of Rt losses mass at first two stages with a molecular rearrangement relating to the loss of water molecules [32].

In TGA analysis rutin's first peak at ~180 °C was related to the phase transition (melting) and also to the molecular rearrangement of its polymorphic structure [34]. Secondary peaks at ~215 °C showed existence of boiling process.

On the TG curve of pure β-CD between 30 and 125 °C, there was an endothermic peak. The correspondence of this peak for dTG was at temperature of 96 °C. This stage of thermal decomposition was related to the dehydration with 10.9 % of water mass loss [35]. On the TGA curves for PM both β-CD and Rt melting peaks were observed verifying Rt still existed as an uncomplexed guest in the samples. The inclusion complex generally did not show the individual melting peak of guest compound, showing that the guest molecule was encapsulated within the CD [35].

The degradation temperature for the β–CD was found as 314 °C and the situation of peak could be explained by the effect of the substituent -*due to the type of substituent*- on the extensively hydrogen bonded supramolecular structure of CDs.

As seen in Fig. 5, melting behavior of PM was more similar to that of Rt. IC showed similar melting pattern to the melting pattern of the cylodextrin's. DTG results of IC and Rt showed that thermal stability of Rt was enhanced after the inclusion complexation.

There is much research on usage of NMR technique for structural characterization of cyclodextrin and its

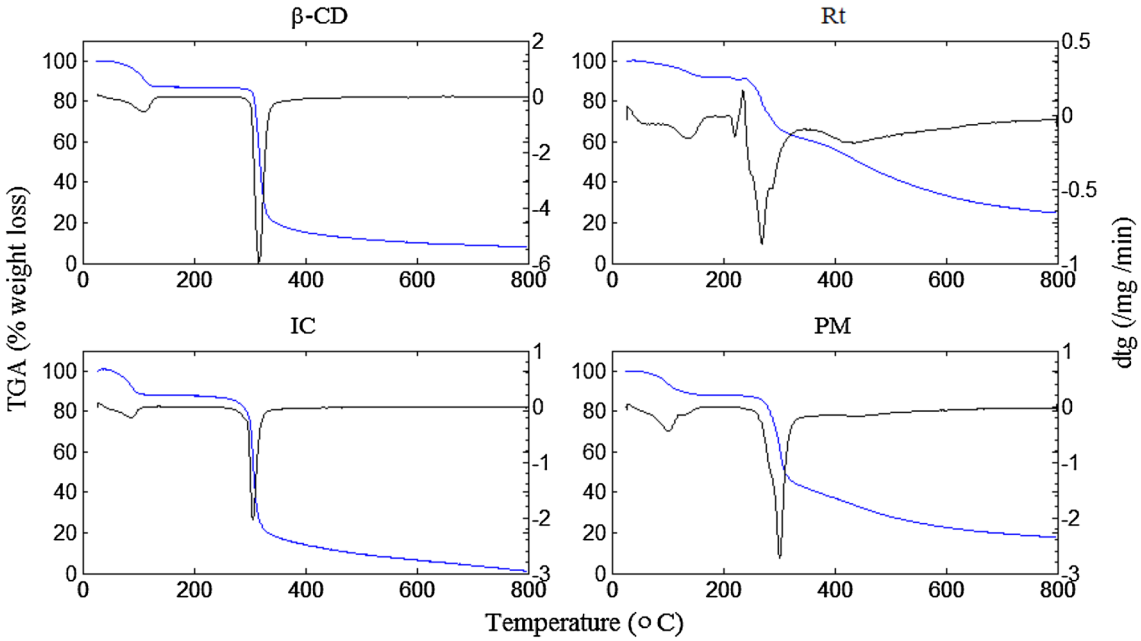

Fig. 5 TGA–dTG curves of β-CD, Rt, IC and PM

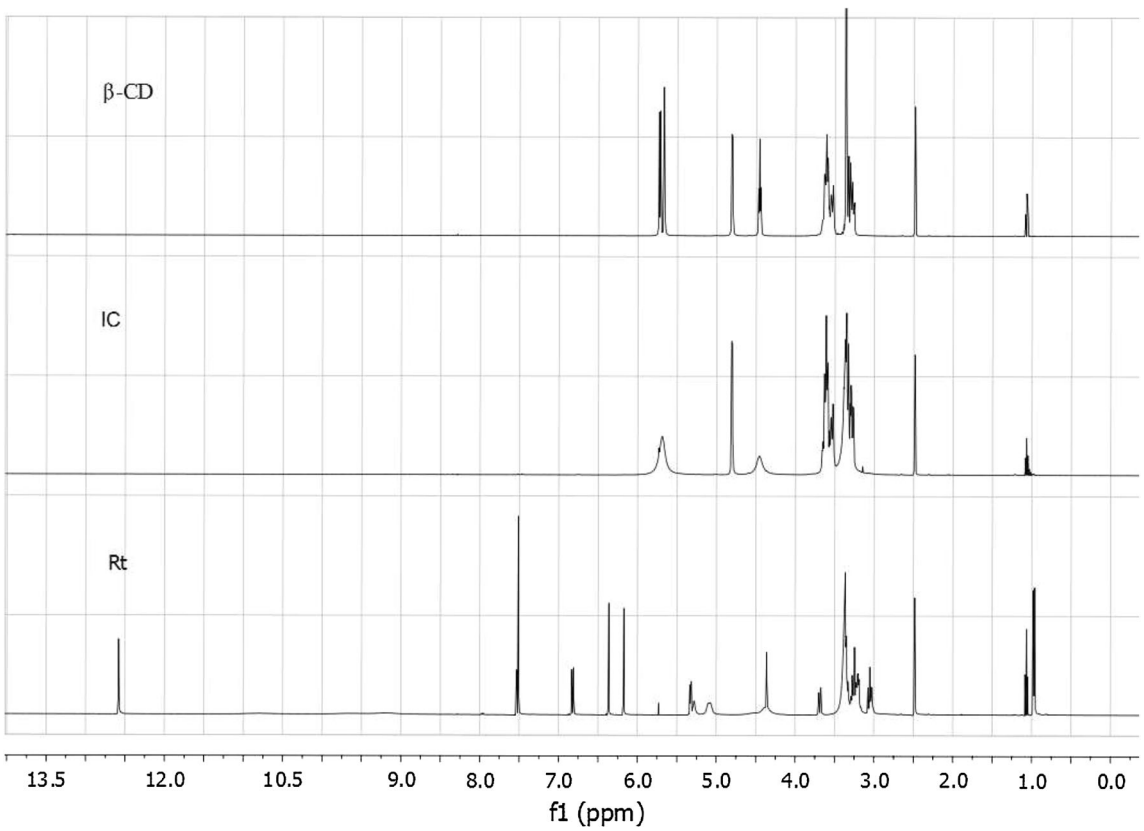

Fig. 6 H-NMR spectra of β-CD, IC and Rt

complexes. For confirming formation of inclusion complex, NMR is one of the most accepted techniques [36].

This technique gives information -whether inclusion complex is formed or not- via the shifts in protons. When a molecule is hold within the cyclodextrin cavity, several of

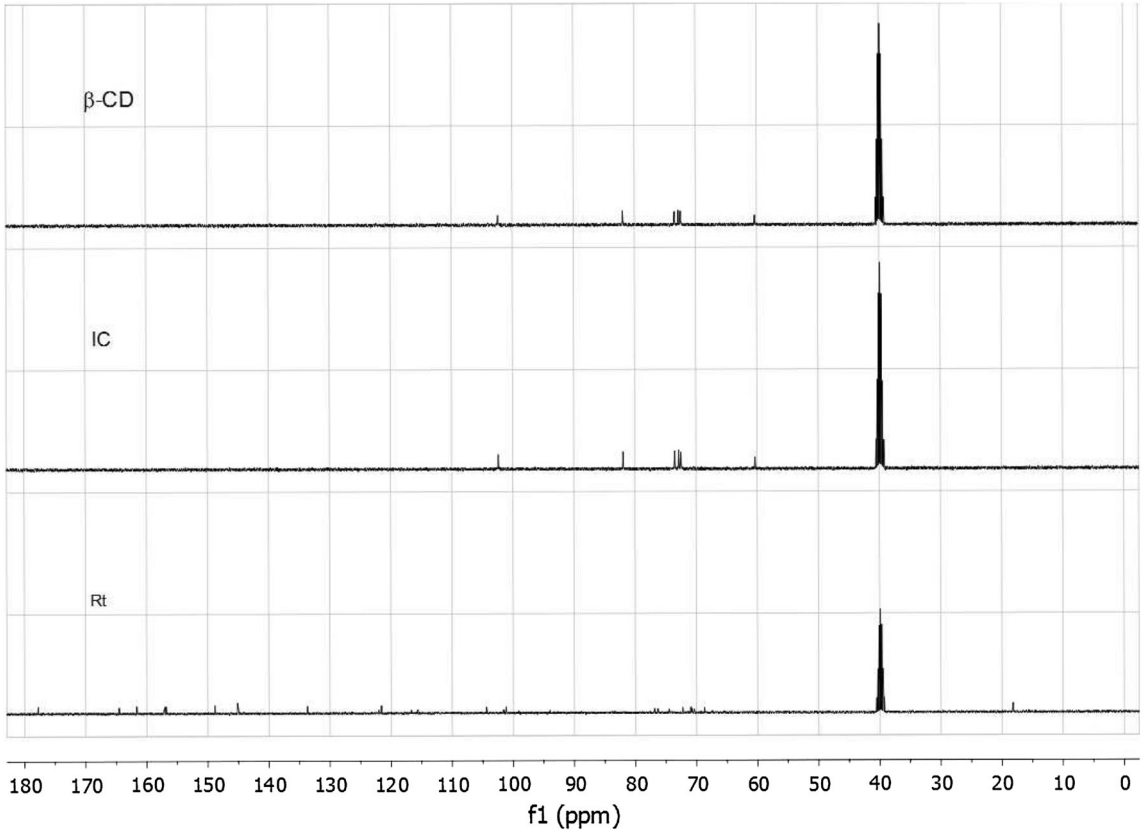

Fig. 7 C-NMR spectra of β-CD, IC and Rt

the protons on the interior of the host and in the protons of the guest-*the part reacting with the cavity*- shifts are observed. According to previous studies, relatively large shift in the interior protons proved formation of inclusion complex [36–38].

The results of NMR analyses are shown in Figs. 6 and 7. H-NMR and C-NMR were applied for Rt, IC and β-CD samples. In both of H-NMR and C-NMR spectra, the characteristic peaks for rutin were observed in spectrum of inclusion complex and its spectrum contained peaks at similar regions with β-CDs. These results showed the formation of inclusion complex between Rt and β-CD.

Electron microscope images of the samples could be seen from Fig. 8. Rt molecules were visualized in the form of column like crystals and β-CD particles showed a diamond shape morphology. In microstructure image of PM, Rt molecules covered surface of β-CD particles and there was not a definite interaction between Rt and β-CD. In SEM image of PM, some part of Rt particles embedded in β-CD and a comparable morphology with pure compounds taken separately, also. In contrast, a profound change was observed in the morphology and shape of IC particles, revealing an apparent interaction in the solid-state. Also, IC presented a homogenous morphology with rectangular prism shaped particles.

Morphology of films

Surface images of SF films (a) Control SF film, (b) Rt, (c) IC and (d) PM loaded film were obtained by SEM magnified at 1,000× see Fig. 9. Also cross section images of the SF films obtained by SEM magnified at 1,000× are given in (Fig. 9). From SEM observations, it could be concluded that at high concentrations of Rt, semi-uniform distribution was observed in SF films while totally uniform distribution was observed for IC loaded SF films. Microscopic phase separation and cracks were not observed for these films. Cross section images of SF films revealed the dense film structures.

Dissolution profile and rutin release

The dissolution characteristics of free Rt and IC of Rt are given in Table 1. More than 70 % of Rt was dissolved at first 20 min when Rt formed complex with β-CD. Dissolution rate of IC was much higher than dissolution rates of physical mixtures and free Rt. Dissolution efficiency percent at 40 min (DE_{40} (%)) were calculated for Rt, IC and PM. The DE_{40} (%) values supported the dissolution characteristics of each compound that were tabulated in Table 1. IC of rutin–β-CD in 1:1 molar ratio showed higher

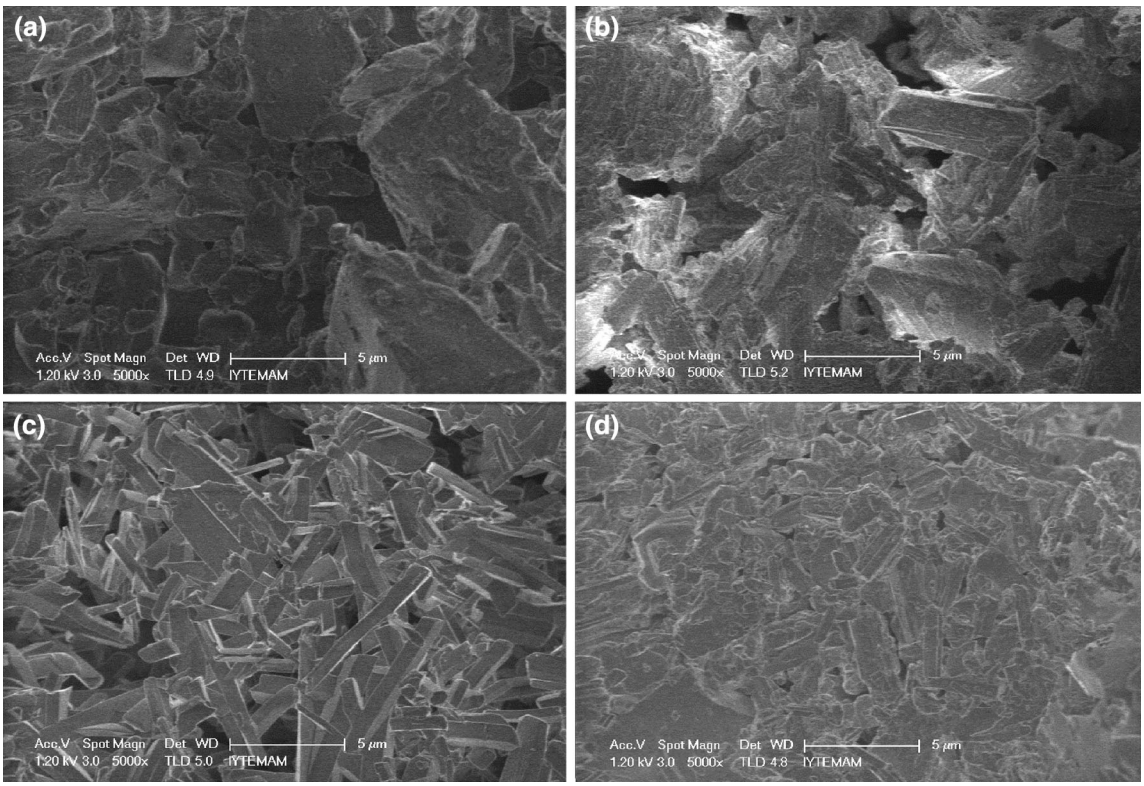

Fig. 8 5,000× magnified SEM images of compounds; β-CD (**a**), Rt (**b**), IC (**c**), PM (**d**)

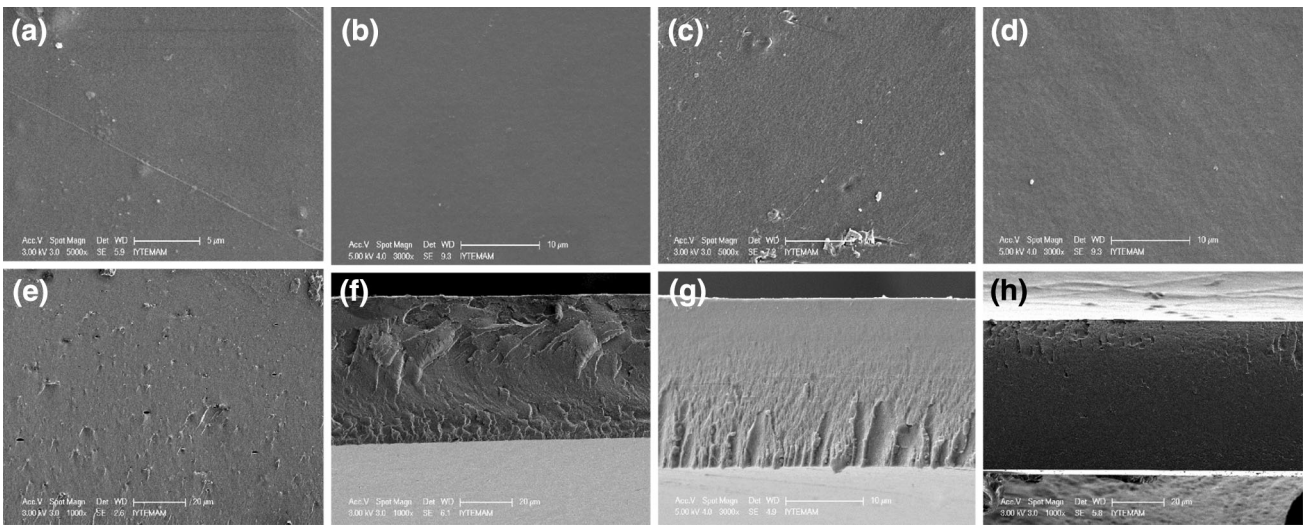

Fig. 9 Surface image of **a** Control SF film, **b** IC film, **c** PM film, **d** Rt film; cross section image of **e** Control SF film **f** IC film **g** PM film **h** Rt film taken with SEM

dissolution efficiency (Fig. 10; Table 4) than the physical mixture and free rutin.

As seen in Figs. 11, 12, 13 almost 50 % of Rt was released from SF film within the first 5 h. The rest of Rt was released within next 19 h completely at a sustained manner. Release behaviour of Rt was mostly the same in both acidic and neutral medium (acetic acid buffer at pH 4.0 and PBS at pH 7.3; 10 mM; 37°C; 100 rpm shaking). As seen in Fig. 11 burst release occurred for IC loaded SF film at the beginning in physiological conditions due to

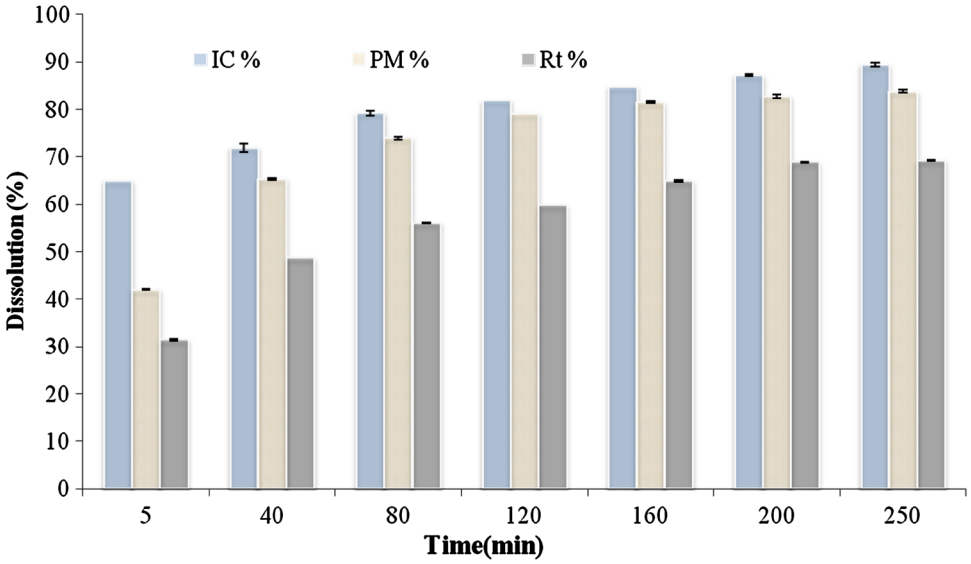

Fig. 10 Percent dissolution profiles of IC, PM, Rt

Table 4 DE $_{40}$ (%) for Rt and Rt-CD binary systems (mean ± SD values; $n = 3$)

Product	DE_{40} (%)
Rt	48.65 ± 0.037
PM of Rt	65.33 ± 0.144
IC of Rt	71.90 ± 0.068

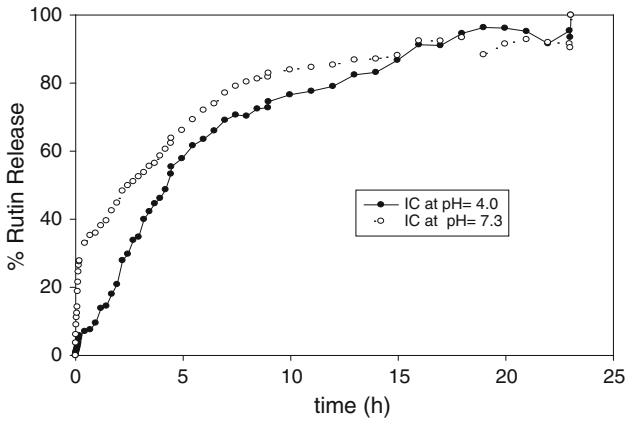

Fig. 11 Release profile for IC of Rt from SF film

high dissolution rates of IC in both neutral and acidic medium. Jug et al. [22] related geometry of the polymer matrix (presence large surface cause fast swelling of rutin) for burst release from films. During release tests, the whole structure of films were preserved both in acidic and neutral release medium, only slight differences in colour of the films were observed. Also, films become crispy and brittle after release period.

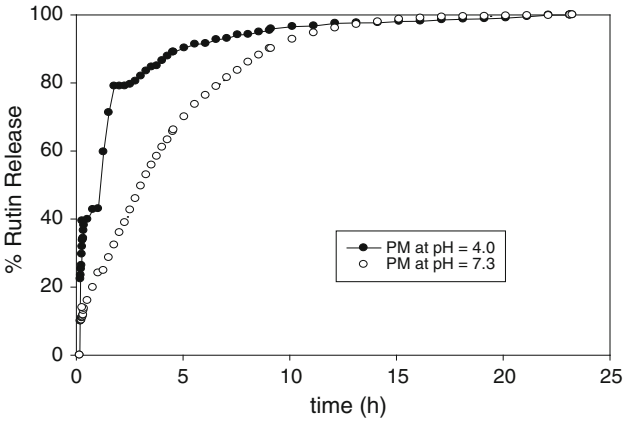

Fig. 12 Release profile for PM of Rt from SF film

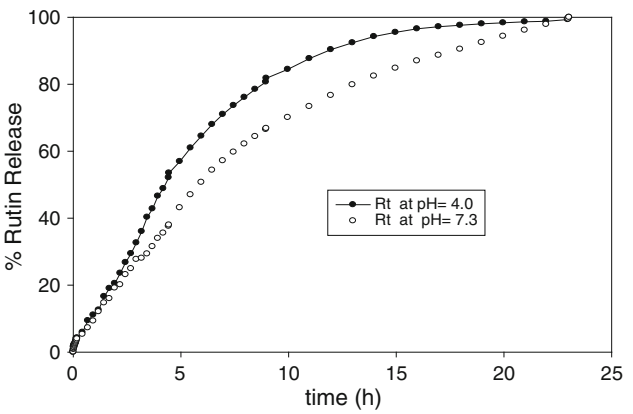

Fig. 13 Release profile for Rt from SF film

Conclusion

Among all of cyclodextrins, β-CD is preferred because of its suitable cavity sizes and low price. Hence, in our present

work, rutin–β-CD solid complex was successfully prepared. Aqueous solubility of Rt was increased as a result of the inclusion complex formation. Improvement in the hydrophilicity of Rt by CD caused to the enhancement of dissolution rate. The calculated solubility energies showed the energy needed to solubilise in water was highest in free Rt and lowest for IC. Analysis of the complexes by X-ray diffraction, DSC and FT-IR methods showed considerable interaction of β-CD with Rt. Results of dissolution profile of Rt, PM and IC showed that addition of CD had an increasing effect on solubility rate and amount. Solid inclusion complexes exhibited higher dissolution efficiencies than their corresponding physical mixtures. Silk fibroin based films were prepared and loading of Rt, PM and IC into silk fibroin based films was carried out successfully. By release tests it was investigated that most of the Rt-*independent from the form, whether free or complexed*-released from SF films within the first 5 h (burst release occurs) and the rest of it released slowly within 24 h. Electron microscope analyses showed that films have a homogenous and dense morphology. Consequently, silk fibroin can be used to load natural compounds in the form of films.

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