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Study of freeze-dried quercetin–cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis

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Abstract

The inclusion behavior of 2-hydroxypropyl β-cyclodextrin (HPβCD) and β-cyclodextrin (βCD), in solution and solid-state was studied towards a poorly water-soluble bioflavonoid, quercetin (QURC), chemically 3,3′,4′,5′,7-pentahydroxy flavone. Drug-cyclodextrin solid systems were prepared by freeze-drying. Phase solubility study was used to evaluate the complexation in solution, of two cyclodextrins, i.e., βCD and HPβCD. The stoichiometry and stability constants of QURC-βCD (1:1 and 402 M^{-1}) and QURC-HPβCD (1:1 and 532 M^{-1}) complexes were calculated by phase solubility method. The formation of inclusion complexes with βCD and HPβCD in the solid-state were confirmed by infrared spectroscopy, differential scanning calorimetry, X-ray diffractometry, and scanning electron microscopy (SEM).
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Keywords: Quercetin; Cyclodextrin; Drug solubilization; Stability constant; Differential scanning calorimetry; Fourier transform infrared spectroscopy; X-ray diffractometry; Scanning electron microscopy

1. Introduction

Flavonoids have a broad pharmacological profile such as antilipoperoxidant [1] and anti-inflammatory [2] properties and the ability to exert anticancer and chemopreventive activities [3,4]. Quercetin, 3,3′,4′,5′-7-pentahydroxy flavone, a polyphenolic flavonoid, extremely hydrophobic in nature, is a component of onion. Quercetin shows several biological effects including a strong inhibitory effect on the growth of several human and animal cancer cell lines [5,6] and enhances the antiproliferative effect of cisplatin both in vitro and in vivo [7]. In spite of this wide spectrum of pharmacological properties, its use in pharmaceutical field is limited by its low aqueous solubility. In recent years, cyclodextrin complexation has been successfully used to improve solubility, chemical stability and bioavailability of a number of poorly soluble compounds. The β-cyclodextrin (βCD) is α-1,4-linked cyclic oligosaccharide composed of seven D-glucopyranose units with a relatively hydrophobic central cavity [8]. However, it is known that the application of β-cyclodextrin in the pharmaceutical field is limited by its rather low aqueous solubility, which led to a search for more soluble derivatives of cyclodextrins [8,9]. Recently, various hydrophilic, hydrophobic and ionic cyclodextrin derivatives have been successfully utilized to extend physicochemical

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properties and inclusion capacity of natural cyclodextrin [10,11]. Hydrophilic cyclodextrins can modify the rate of drug release for the enhancement of drug absorption across biological barriers. Amorphous cyclodextrins such as 2-hydroxypropyl β-cyclodextrin is useful for inhibition of polymorphic transition and crystallization rates of poorly water-soluble drugs during storage, which can consequently maintain the higher dissolution characteristics and oral bioavailability of the drugs [12].

In the present study solubilization of quercetin was achieved by complexation with natural β-cyclodextrin and its 2-hydroxypropyl derivative. The stoichiometry and stability constant of the complexes were determined by evaluating drug-cyclodextrin interactions in solution using phase solubility analysis. Drug-cyclodextrin solid systems were prepared by method of freeze-drying. Additional information on the complexing efficacies of the two cyclodextrins toward quercetin was obtained by differential scanning calorimetry, FT-IR, powder X-ray diffractometry and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

Quercetin (QURC) was purchased from S.D. Fine Chemicals (Mumbai, India) β-cyclodextrin and 2-hydroxypropyl β-cyclodextrin were kindly provided by S.A. Chemicals (Mumbai, India). All other reagents and solvents used were of analytical grade.

2.2. Phase solubility studies

Solubility measurements were performed according to Higuchi and Connors [13]. Excess amounts of drug were added to 10 ml of water or aqueous solution of CDs (0.003–0.015 M concentration range) in 25 ml stoppered conical flasks and shaken at 25 ± 0.5 °C. At equilibrium after 2 days, aliquots were withdrawn, filtered (0.45 μm pore size) and spectrophotometrically assayed for drug content at 372 nm (Milton Roy UV-Vis Spectrophotometer 1201). Each experiment was carried out in triplicate (coefficient of variation (CV) <3%). The apparent binding constants of the QURC-CD complexes were calculated from the slope and intercept of the straight lines of the phase solubility diagrams according to the following equation:

$$\mathrm{Kc} = \frac{\text{slope}}{\mathrm{So}(1 - \text{slope})}$$

2.3. Preparation of solid inclusion complexes

2.3.1. Freeze-drying method

The method followed for inclusion complexation of quercetin with βCD and HPβCD is described by Nagai et al. [14]. Solid-state quercetin complexes with βCD and HPβCD in 1:1 molar ratios were prepared. Quercetin (0.302 g), βCD (1.135 g) and HPβCD (1.550 g) were accurately weighed and dissolved in distilled water (70 ml). To this aqueous solution further, 25% ammonia (1.5 ml) was added to dissolve the quercetin. The whole solution was stirred on magnetic stirrer for 2 h. The solution was frozen overnight and then lyophilized over period of 30 h using freeze-drier, Lobaconco freeze dry system, Freezone 4.5 at 40 ± 1.0 °C with 50 mbar vacuum for 24 h. The dried *powder* was passed through sieve (60#) and stored in a dessicator until further evaluation.

2.4. Preparation of physical mixtures

Physical mixtures (PM) were obtained by pulverizing in a glass mortar and carefully mixing an accurately weighed (1:1 molar ratio) amounts of flavonoid and cyclodextrins.

2.5. Differential scanning calorimetry (DSC)

A Perkin-Elmer DSC model 7 was used for recording DSC thermograms of the quercetin raw material, inclusion complexes prepared by freeze-drying as well as the physical mixtures. Samples (2–8 mg) were accurately weighed using a Sartorius 4503 electronic microbalance and heated in closed aluminium crimped cells at a rate of 10 °C min^{-1} between 30 and 300 °C temperature range under a nitrogen flow of 40 ml min^{-1}. Reproducibility was checked by running the sample in triplicate.

2.6. Fourier transform infrared (FT-IR) spectroscopy

Fourier transform IR spectra were recorded on a Jasco FT-IR-281 spectrophotometer. The spectra were

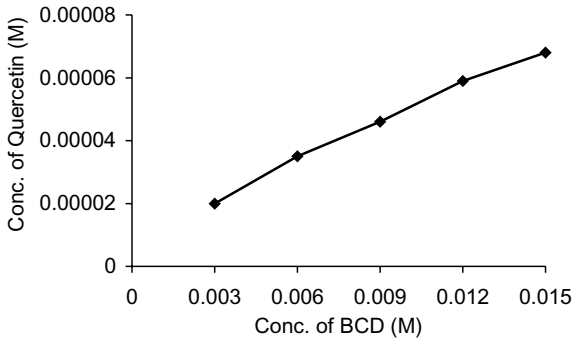

Fig. 1. Phase solubility diagram of QURC-βCD system.

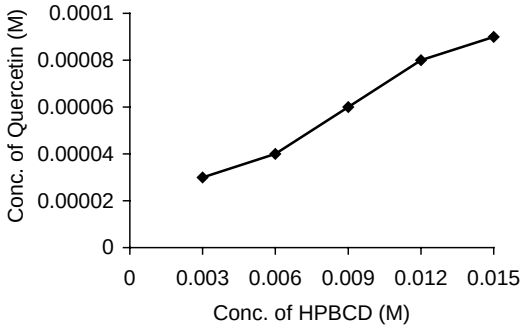

Fig. 2. Phase solubility diagram of QURC-HPβCD system.

recorded for quercetin, βCD, HPβCD, physical mixtures and their freeze-dried complexes. Samples were prepared in KBr disks prepared with a hydrostatic press at a force of 5.2 T cm^{-2} for 3 min. The scanning range was 450–4000 cm^{-1} and the resolution was 1 cm^{-1}.

2.7. X-ray powder diffractometry

X-ray powder diffraction patterns were recorded on a Philips PW 17291 powder X-ray diffractometer using Ni-filtered, Cu Kα radiation, a voltage of 40 kV and a 25 mA current. The scanning rate employed was 1° min^{-1} over the 10–40° 2θ range. The XRD patterns of quercetin raw material, inclusion complexes as well as the physical mixtures were recorded.

2.8. Scanning electron microscopy (SEM) analysis

SEM analysis was carried out using a Jeol JSM-840 scanning electron microscope. Prior to examination,

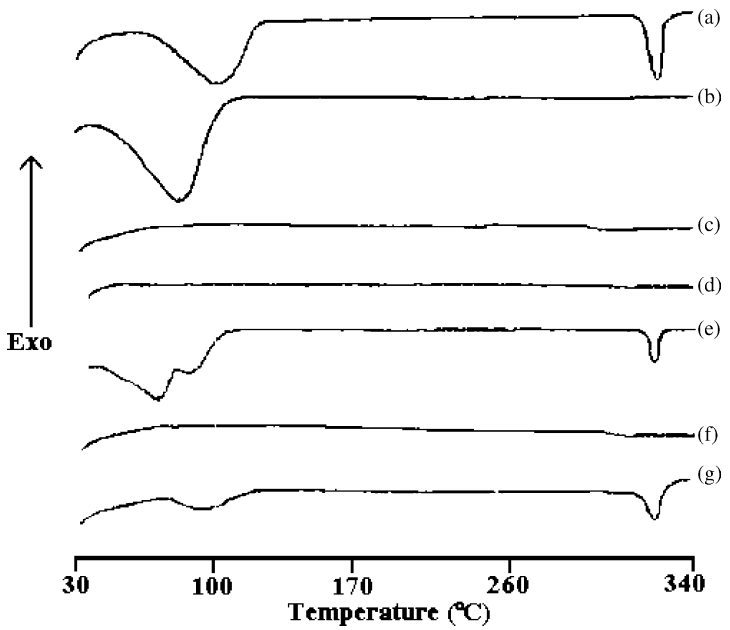

Fig. 3. DSC curves of QURC (a); βCD (b); HPβCD (c); QURC-βCD freeze-dried product (d); QURC-βCD equimolar physical mixture (e); QURC-HPβCD freeze-dried product (f); QURC-HPβCD equimolar physical mixture (g).

samples were gold sputter-coated to render them electrically conductive.

3. Results and discussion

3.1. Solubility studies

Phase solubility diagrams of QURC-βCD and QURC-HPβCD are shown in Figs. 1 and 2, respectively. Phase solubility diagrams obtained with βCD and HPβCD showed a linear relationship between the amount of QURC solubilized and the concentration of cyclodextrin in solution (AL type diagram). According to Higuchi and Connors theory [13], this may be attributed to the formation of soluble 1:1 QURC-cyclodextrin inclusion complexes. Stability constant obtained for QURC was in the rank order of HPβCD (532 M^{-1}) > βCD (402 M^{-1}).

3.2. Solid-state studies

3.2.1. Differential scanning calorimetry

The thermal curves of pure components and of the different drug-cyclodextrin systems are shown in Fig. 3. Quercetin is a dihydrate molecule and in the DSC curve it showed a broad endothermic peak (T_{peak} = 101 °C) as it becomes anhydrous and a melting endotherm (T_{onset} = 322.8 °C; T_{peak} = 326.2 °C). Liberation of crystal water from βCD (14.5% as mass fraction) was observed as a broad

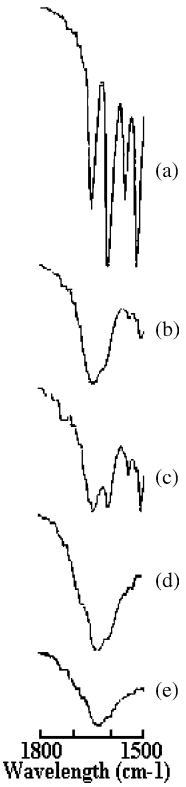

Fig. 4. FT-IR spectra of QURC (a); QURC-βCD equimolar physical mixture (b); QURC-HPβCD equimolar physical mixture (c); QURC-βCD freeze-dried product (d); QURC-HPβCD freeze-dried product (e).

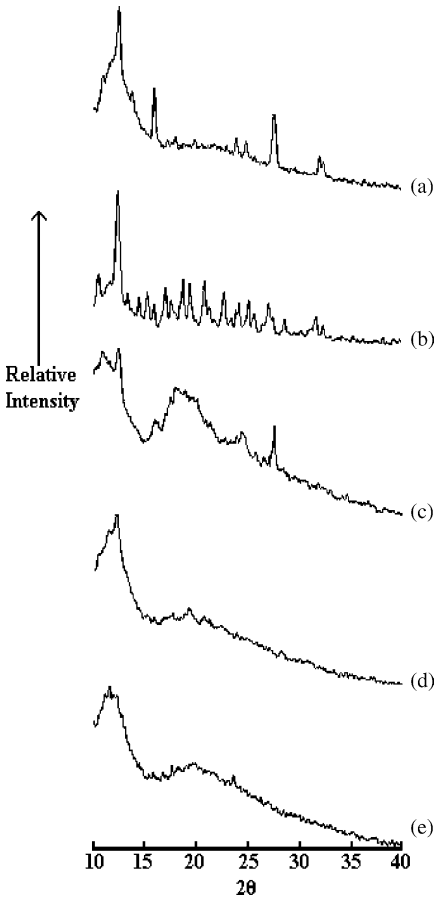

Fig. 5. XRD spectra of QURC (a); QURC-βCD equimolar physical mixture (b); QURC-HPβCD equimolar physical mixture (c); QURC-βCD freeze-dried product (d); QURC-HPβCD freeze-dried product (e).

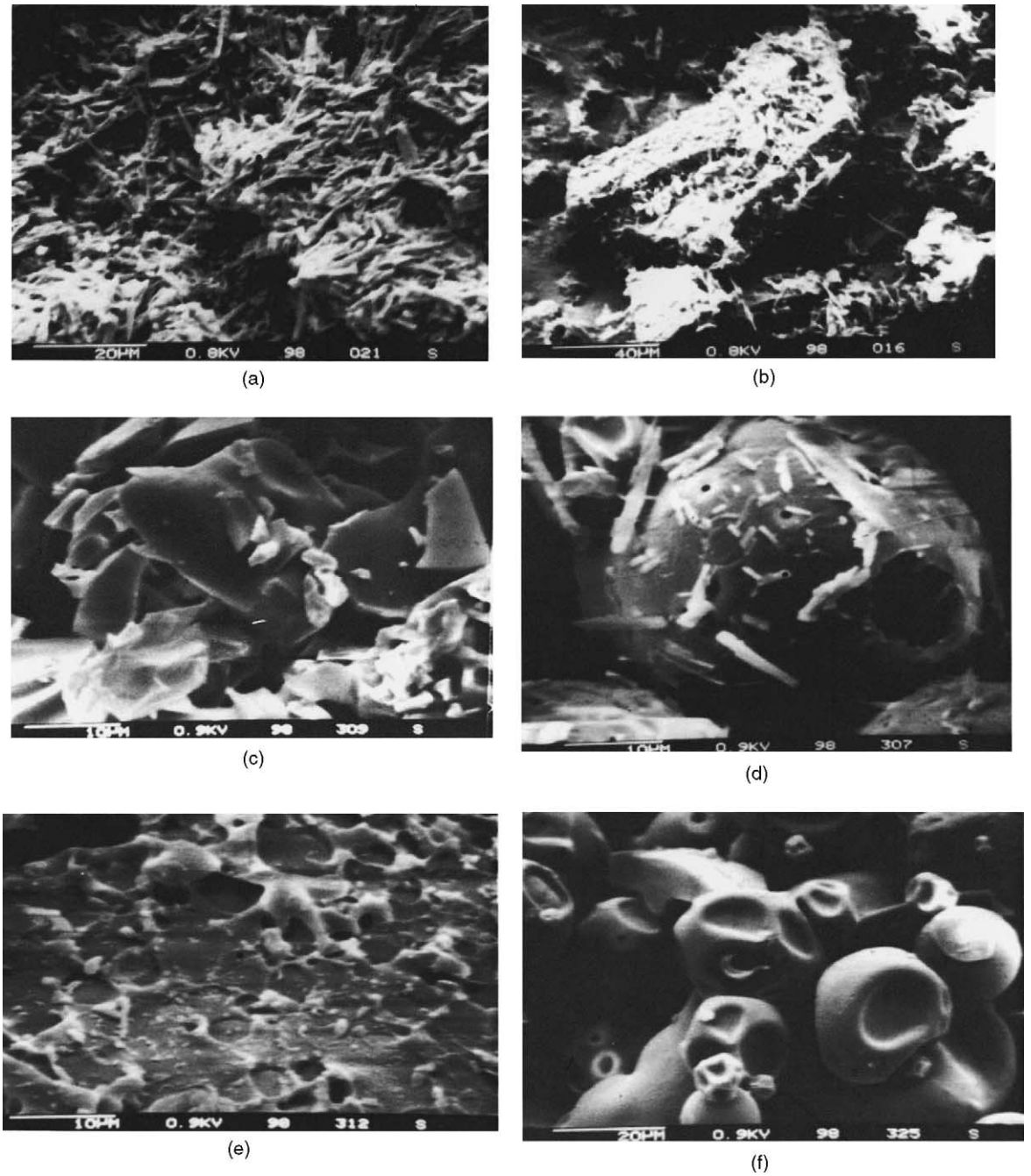

Fig. 6. SEM photographs of QURC (a); QURC-βCD physical mixture (b); QURC-βCD *freeze-dried complex* (c); QURC-HPβCD physical mixture (d); QURC-HPβCD *freeze-dried complex* (e); HPβCD (f); βCD (g).

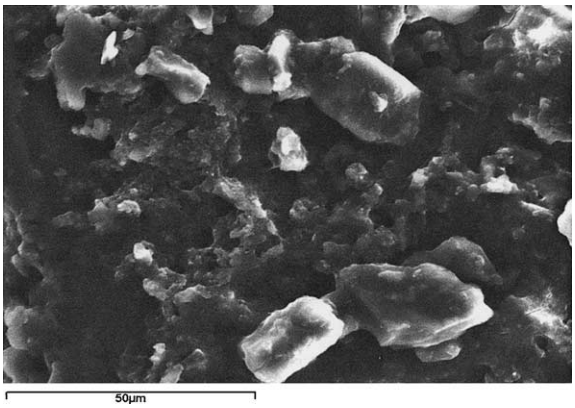

Fig. 6. (*Continued*).

endothermal peak at around 100 °C. Broader endotherm was associated with water loss of 11.9% as mass fraction from amorphous HPβCD. The characteristic, well recognizable thermal profile of the drug appeared at the temperature corresponding to its melting point in the physical mixtures of drug with both, βCD and HPβCD. The complete disappearance of the drug endothermal peak was instead observed for systems obtained by freeze-drying. This phenomenon can be assumed as proof of interactions between the components of the respective binary systems [15]. This can be considered as indicative of drug amorphization and/or inclusion complex formation.

3.2.2. Infrared spectroscopy

Infrared spectra of QURC, as well as those of its solid systems with CDs, are presented in Fig. 4. Drug crystals show a characteristic carbonyl absorption band at 1664.72 cm^{-1}, assigned to aromatic ketonic carbonyl stretching. The FT-IR spectra of drug–CD complex were compared to the physical mixtures and pure drug. In the case of freeze-dried products, in particular, the characteristic aromatic carbonyl-stretching band of drug appeared shifted to 1637.71 and 1635.78 cm^{-1} for QURC-βCD and QURC-HPβCD complexes, respectively, along with reduced intensity of the same band. Changes in the characteristic bands of pure drug confirm the existence of the complex as a new compound with different spectroscopic bands [16].

3.2.3. X-ray diffractometry

Fig. 5 shows the X-ray diffraction patterns of QURC and corresponding complexes with CDs. In the X-ray diffractogram of QURC powder, sharp peaks at a diffraction angle of 2θ 12.48, 15.86, 23.88, 24.88° are present and it suggests that the drug is present as a crystalline material. Drug crystallinity peaks were still detectable in the respective physical mixtures with βCD and HPβCD. A total drug amorphization was instead induced by freeze-drying where X-ray diffraction patterns of QURC-CD systems were characterized only by large diffraction peaks in which it is no longer possible to distinguish the characteristic peaks of flavonoid. These results, confirm that QURC is no longer present as a crystalline material and its CD solid complexes exist in the amorphous state.

3.2.4. Scanning electron microscopy

From SEM analysis, QURC was seen as needle-like crystals that formed aggregates. HPβCD is observed as “shrinked” cylindrical spheres, whereas βCD appeared as irregularly shaped crystals. The physical mixtures showed particles of HPβCD and βCD embedded with QURC particles and a comparable morphology with pure compounds taken separately. In contrast, a drastic change in the morphology and shape of particles was observed in 1:1 freeze-dried products of both, HPβCD and βCD, revealing an apparent interaction in the solid-state (Fig. 6).

4. Conclusions

Through complexation with the two cyclodextrins, βCD and HPβCD, the aqueous solubility of QURC has been improved substantially (up to 10-fold) in neutral aqueous solutions. All the data obtained from FT-IR, DSC, X-ray diffraction and SEM studies showed that it is possible to obtain an inclusion complex with a stoichiometry of 1:1, in the solid-state and in aqueous solution, with an overall complexing ability that is slightly greater for the HPβCD derivative. Thus, βCD and its derivatives may be useful in improving the dissolution and the bioavailability of quercetin in pharmaceutical formulation.

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