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Preparation and Characterization of Quercetin and Rutin Cyclodextrin Inclusion Complexes

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ABSTRACT The objective of the present study is to prepare and characterize cyclodextrin inclusion complexes of quercetin and rutin to improve their aqueous solubility and dissolution properties. Inclusion complexes of quercetin and rutin with ß-cyclodextrin (ß-CD) and hydroxyl propyl-ß-cyclodextrin (HP-ß-CD) were prepared by kneading and coevaporation methods. Characterization of inclusion complexes was done by phase solubility analysis and was supported by X-ray powder diffractometry (XRD), differential scanning calorimetry (DSC), and Fourier-transform infra red spectroscopy (FT-IR) analysis. Inclusion complexes exhibited higher rates of dissolution than the corresponding physical mixtures and pure drug. Higher dissolution rates were observed with HP-ß-CD kneaded complexes in comparison to the products with ß-CD.

KEYWORDS Phase solubility, Quercetin, Rutin, CD, Dissolution, Physicochemical characterization

INTRODUCTION

Bioflavonoids are a group of polyphenolic compounds which are the most common biological constituents in plants and are found in many fruits, vegetables, grains, nuts, leaves, and flowers (Degroot & Rauen, 1998). Bioflavonoids have attracted attention due to their various promising biological activities including anti-inflammatory (Read, 1995), anti-allergic (Bronner & Landry, 1985), anti-viral (Kaul & Middleton, 1985), anticancer (Hertog et al., 1993a), and antioxidant (Allan & Miller, 1996) properties. Particular attention is being devoted to quercetin and rutin, two bioflavonoids which form a pair and have significant therapeutic potential in treating capillary fragility (Ljungman et al., 1996), cancer (Hertog et al., 1993a), cardio-vascular disorders (Hertog et al., 1993b), and diabetic complications (Monograph on Quercetin, 1998; Annapurna et al., 2005).

Unfortunately, though they have good therapeutic potential, these bioflavanoids are poorly soluble in water. The poor aqueous solubility of the drugs often leads to variable and poor bioavailability. Their oral absorption is limited by their decreased dissolution rate. Any attempt to enhance the dissolution rate

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would improve their absorption and bioavailability (Peter et al., 1997).

Cyclodextrins have been playing a very important role in the formulation of poorly water soluble drugs by improving apparent drug solubility and/or dissolution through inclusion complexation or solid dispersion by acting as hydrophilic carriers. Cyclodextrins, as a result of their ability to form in situ inclusion complexes in dissolution medium, can enhance drug dissolution even when there is no complexation in the solid state (Challa, 2005).

Cyclodextrin inclusion complexes have been shown to improve the stability, solubility, dissolution rate, and bioavailability of the drugs (Loftsson & Brewster, 1996; Rajewski & Stella, 1996, 1997). CD complexation was attempted with many drugs like rutin (Haiyun et al., 2003; Calabro et al., 2005), quercetin (Pralhad & Rajendrakumar, 2004; Zheng et al., 2005) piroxicam (Guo et al., 2003) and the resultant complexes were evaluated for their inclusion interactions.

Hydrophilic cyclodextrins can modify the rate of drug release and cause the enhancement of drug absorption across biological barriers. Amorphous cyclodextrins such as HP-ß-CD are useful for inhibition of polymorphic transition and crystallization rates of poorly water soluble drugs during storage. The complexes can consequently maintain higher dissolution characteristics and oral bioavailability of the drugs (Liu & Zhu, 2006).

Hence, in the present study, preparation and evaluation of both quercetin (aglycone) and rutin (glycoside) inclusion complexes with β-CD and HP-ß-CD was attempted with an aim to improve their aqueous solubility and dissolution rate.

MATERIALS AND METHODS

Materials

Rutin was purchased from Sigma Chemicals, quercetin was a gift sample from AIE chemicals (USA) and ß-CD and HP-ß-CD were kindly provided by SA chemicals, Mumbai. All other chemicals and reagents were of analytical grade and double distilled water was used throughout the study (see Fig. 1).

Phase Solubility studies

Phase solubility studies were performed according to the method reported by Highuchi & Connors

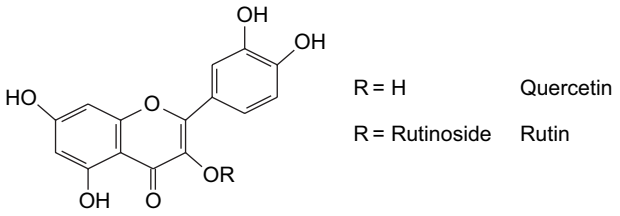

FIGURE 1 **Structure of Rutin and its Aglycone Quercetin. If R is Rutinoside, then the structure stands for Rutin. If R is Haglycone, then the structure stands for Quercetin.**

(1965). Quercetin/rutin in amounts that exceeded their solubilities were taken and added to distilled water containing various concentrations of cyclodextrins (2.5 mM to 20 mM). The suspensions were shaken at a temperature of 28°C ± 1°C for 48 hr on a rotary flask shaker to reach the equilibrium. Subsequently the aliquots were withdrawn at 1 hr intervals and the samples were filtered using a 0.45 μm nylon disc filter and analyzed by UV spectrophotometer (quercetin at 370 nm and rutin at 359 nm) against the blanks prepared with the same concentration of cyclodextrins in water. Shaking was continued until three consecutive estimations were equivalent. The solubility experiments were conducted in triplicate.

The apparent stability constant (K_c) was calculated from the phase solubility diagrams using the following equation,

$$K_C = \frac{\text{Slope}}{S_o(1-\text{slope})}$$

The slope was obtained from the initial straight line portion of the plot of quercetin/rutin concentration against cyclodextrin concentration and from that the equilibrium solubility of quercetin/rutin in water was calculated.

Preparation of Solid Inclusion Complexes

Physical mixtures were prepared by homogeneous blending of previously sieved and weighed quercetin/rutin cyclodextrins in a mortar in 1:1 and 1:2 molar ratios.

Kneading Method

The solid quercetin/rutin complexes with cyclodextrins in a molar ratio of 1:1 and 1:2 were prepared by kneading method using ethanol–water (1:1 v/v) as a solvent. The calculated amounts of quercetin/rutin and

cyclodextrin were weighed and triturated with a small amount of ethanol–water and the slurry was kneaded thoroughly for about 45 min and then dried at 45°C. The dried mass was pulverized and sieved through a 100 mesh sieve. It was then stored in a desiccator.

Coevaporation Method

The aqueous solution of cyclodextrin was added to alcoholic solution of quercetin/rutin. The mixture was stirred for 1 hr and evaporated at a temp of 45°C until it dried. The dried mass was pulverized and sieved through a 100 mesh sieve. It was then stored in a desiccator.

Powder X-ray Diffraction Studies

The powder X-ray diffraction patterns were recorded using a Shimadzu XRD 6000 with Cu as anode material and crystal graphite monochromator applied at a voltage of 40 kV. All the samples were analyzed in the 2θ angle range of 5° to 50°.

Differential Scanning Calorimetry

The DSC measurements were performed using Mettler Toledo star system e 822 differentialscanning calorimeter. Accurately weighed samples (3–9 mg) were sealed in separate aluminum pans before heating at a scanning rate of 10°C/min over the temperature range of 50–350°C for quercetin/rutin. An empty aluminum pan was used as a reference sample.

Fourier Transform-Infra Red Spectroscopy

FT-IR spectra were recorded on a Perkin-Elmer spectrophotometer. Samples were prepared in KBr disks prepared with a hydrostatic pressure at a force of 5.0 ton cm^{-2} for 2 min. The scanning range was 450–4000 cm^{-1} and the resolution was 1 cm^{-1}.

Dissolution Rate Studies

The dissolution rate studies were performed using a USP dissolution rate test apparatus which uses a paddle method. Samples corresponding to 20 mg of drug were taken. The dissolution medium was 900 mL of distilled water containing 0.75% SLS. The stirring speed was 50 rpm and the temperature was 37°C ± 1°C. Samples (5 mL) were withdrawn at various time intervals, filtered using a 0.45 μm nylon disc filter and were analyzed by a UV spectrophotometer as described earlier. The dissolution profiles were evaluated on the basis of dissolution efficiency (DE; Khan, 1975) at 30 min.

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

Statistical Analysis

Statistical analysis was performed to study the significance of the results of dissolution tests comparing pure drug and quercetin/rutin complexes with ß-CD/HP-ß-CD. Different complexation methods were also compared by two-way analysis of variance procedure.

RESULTS AND DISCUSSION

Phase Solubility Studies

The phase solubility diagrams for the complex formation between quercetin/rutin with ß-CD and HP-ß-CD are presented in Fig. 2a,b and Table 1.

These plots showed that the aqueous solubility of the drug increases linearly as a function of cyclodextrin concentration. It is clearly observed that the solubility diagrams of quercetin and rutin in the presence of ß-CD or HP-ß-CD can be classified as A_L type. This may be attributed to the formation of soluble 1:1 Drug–CD inclusion complexes. Stability constants obtained for quercetin were in the rank order of HP-ß-CD (272 M^{-1}) >ß-CD (251 M^{-1}) and for rutin were HP-ß-CD (341 M^{-1})

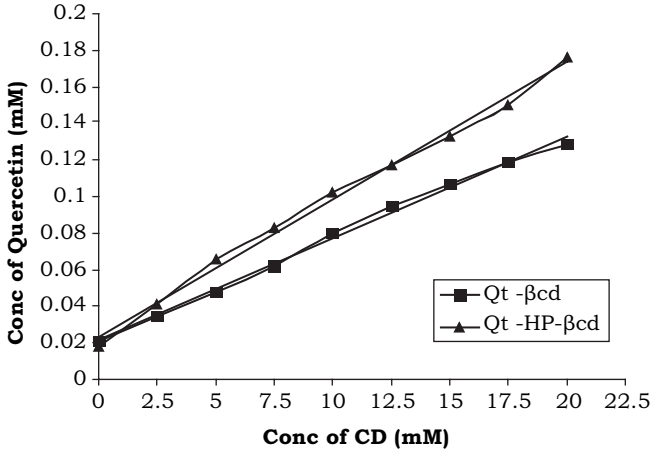

FIGURE 2A **Phase Solubility Diagrams of Quercetin-CD Systems in Water (*n* = 3) Qt - Quercetin.**

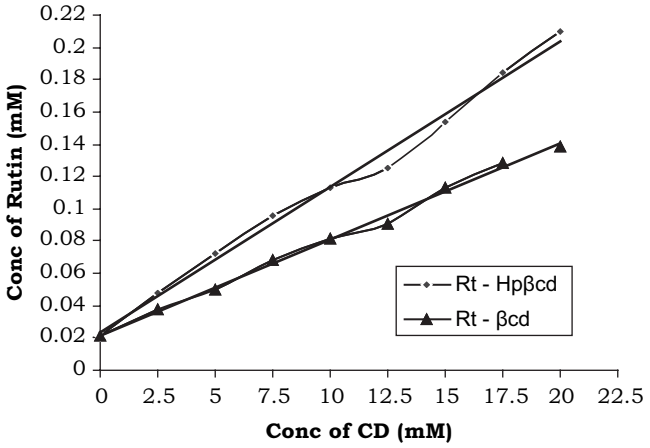

FIGURE 2B **Phase Solubility Diagrams of Rutin-CD Systems in Water (n = 3), Rt - Rutin.**

TABLE 1 **Summary of Quercetin/Rutin-CD Phase Solubility Studies**

CD	Type of phase solubility diagram	Stability constant (M^{-1})	
		Quercetin	Rutin
ß-CD	A_L	251 (r^2 = 0.998)	260 (r^2 = 0.973)
HP-ß-CD	A_L	321 (r^2 = 0.996)	341 (r^2 = 0.938)

CD-Cyclodextrin.

> ß-CD (260 M^{-1}) which indicated that the quercetin/rutin-CD complexes were adequately stable.

Powder X-ray Diffraction

Powder X-ray diffractometry is a useful method for the detection of cyclodextrin complexation in powder or microcrystalline states (Liu & Zhu, 2006). The diffraction pattern of the complex is supposed to be clearly distinct from that of each of the components. Crystallinity is determined by comparing representative peak heights in the diffraction patterns. The powder XRD pattern of quercetin pure drug shows (Fig. 3) highly crystalline nature as evident from the sharp peaks observed at 14°, 16°, and 27° of 2θ values. Crystallinity peaks were still detectable in the coevaporation and physical mixtures with ß-CD/HP-ß-CD. Fig. 4 shows the X-ray diffraction patterns of rutin and corresponding complexes with CDs. In the X-ray diffractogram of rutin powder sharp peaks at a diffraction angle of 2θ, 11.2°, 14.8°, 16.2°, 26°, are present and it suggests that the drug is present as a crystalline material. Drug crystallinity peaks were still detectable in the coevaporation and physical mixtures with β-CD/HP-ß-CD.

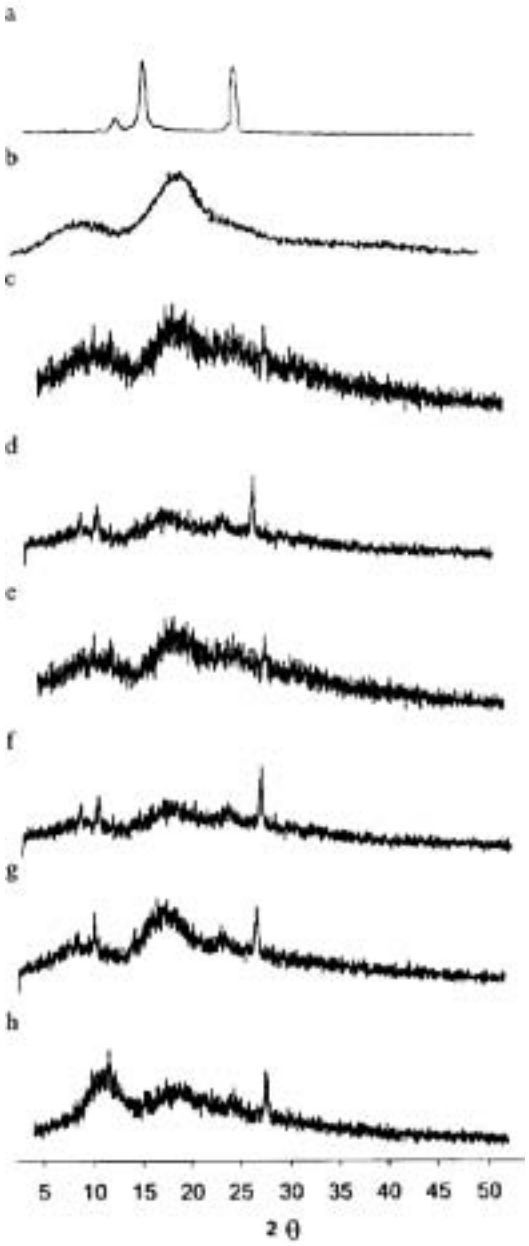

FIGURE 3 **Powder X-ray Diffractogram of Quercetin and Quercetin-HP-ß-CD Binary Systems. (a) Quercetin; (b) HP-ß-CD; (c) Quercetin-HP-ß-CD 1:2 Kn; (d) Quercetin-HP-ß-CD 1:1 Kn; (e) Quercetin-HP-ß-CD 1:2 Co; (f) Quercetin-HP-ß-CD 1:1 Co; (g) Quercetin-HP-ß-CD 1:2 Pm; (h) Quercetin-HP-ß-CD 1:1 Pm.**

A total drug amorphization was instead induced by kneading systems where XRD patterns of quercetin/rutin-CD (1:2) were characterized only by large diffraction peaks in which it is no longer possible to distinguish the characteristic peaks of the flavonoids. These results, confirm that quercetin/rutin are no longer present as a crystalline material and their solid complexes exist in the amorphous state.

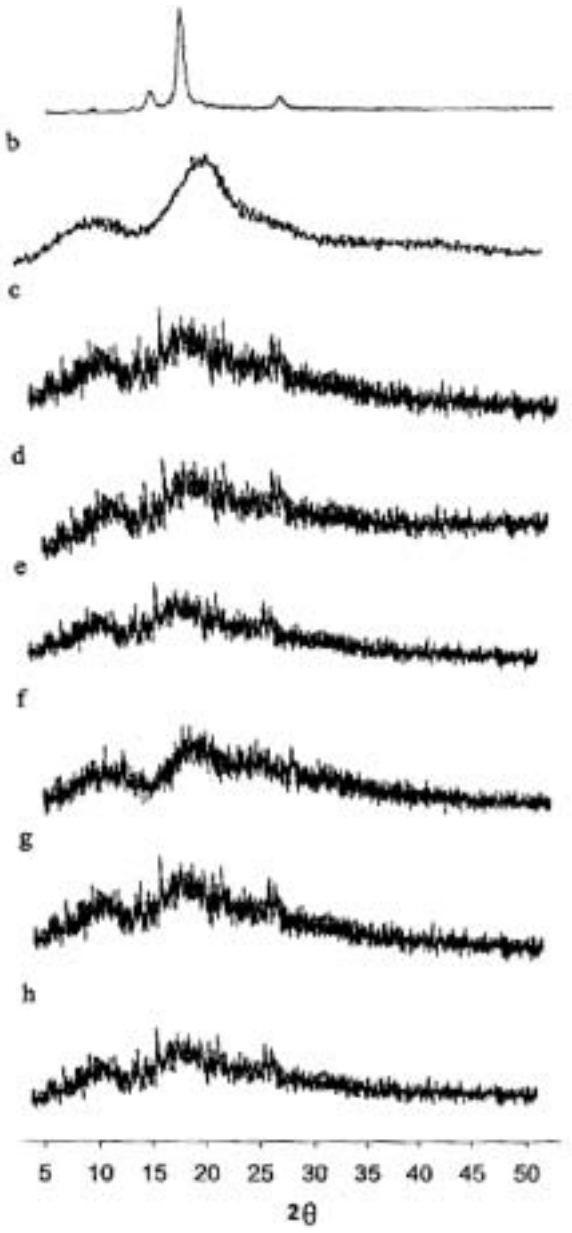

FIGURE 4 **Powder X-ray Diffractogram of Rutin and Rutin-HP-ß-CD Binary Systems. (a) Rutin; (b) HP-ß-CD; (c) Rutin-HP-ß-CD 1:2 Kn; (d) Rutin-HP-ß-CD; 1:1 Kn; (e) Rutin-HP-ß-CD 1:2 Co; (f) Rutin-HP-ß-CD 1:1 Co; (g) Rutin-HP-ß-CD 1:2 Pm; (h) Rutin-HP-ß-CD 1:1 Pm.**

Differential Scanning Calorimetry

DSC can be used for the recognition of inclusion complexes. When guest molecules are embedded in CD cavities or in the crystal lattice, their melting, boiling or sublimation points generally shift to a different temperature or disappear (Buchi et al., 2003). The thermograms of quercetin and its binary systems are shown in Fig. 5 quercetin-HP-ß-CD.

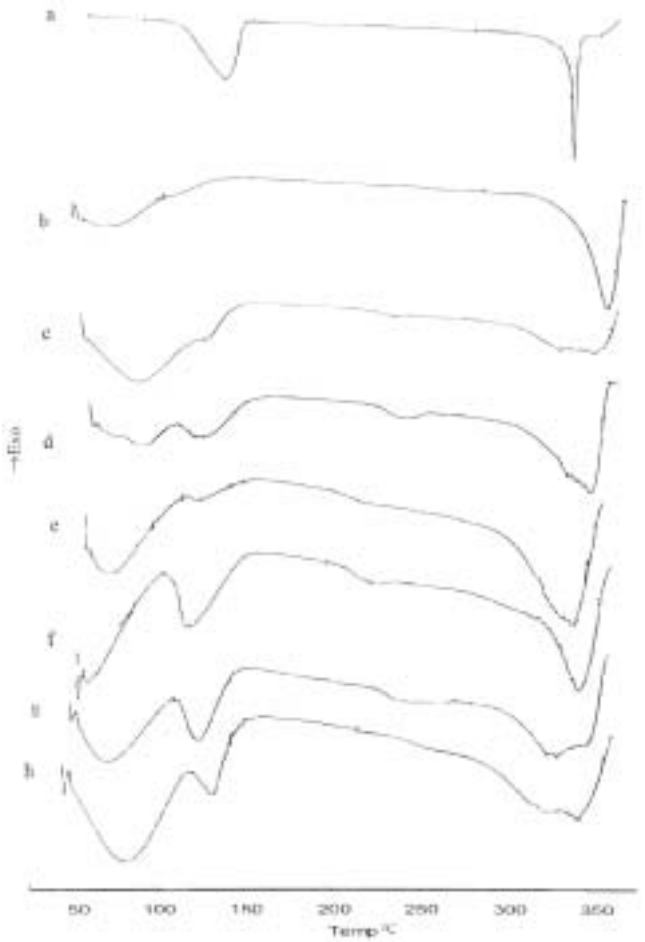

FIGURE 5 **DSC Thermogram of Quercetin and Quercetin-HP-ß-CD Binary Systems. (a) Quercetin; (b) HP-ß-CD; (c) Quercetin-HP-ß-CD 1:2 Kn; (d) Quercetin-HP-ß-CD 1:1 Kn; (e) Quercetin-HP-ß-CD 1:2 Co; (f) Quercetin-HP-ß-CD 1:1 Co; (g) Quercetin-HP-ß-CD 1:2 Pm; (h) Quercetin-HP-ß-CD 1:1 Pm.**

The DSC thermogram of quercetin exhibited a sharp endothermic peak at 129.87°C and 324.5°C indicating the melting point. ß-CD showed a very broad endothermic effect, between 60°C and110°C which attained a maximum around 95°C, corresponding to dehydration process, followed by an irreversible solid–solid phase transition at 216°C and finally, to a degradation process, which took place at around 300°C. The thermogram of HP-ß-CD exhibited a very broad endothermic peak between 60°C and 100°C (maximum at 72°C) corresponding to release of water molecules. For the kneaded mixture (1:2 M) of quercetin and ß-CD/HP-ß-CD, the drug endothermic peak at 129.87°C disappeared and the drug peak at 324.5°C shifted to 305.51°C. Similarly the ß-CD peak shifted from 228.2°C to 217°C. The DSC profile of coevaporated drug-ß-CD/HP-ß-CD mixtures and physical mixtures,

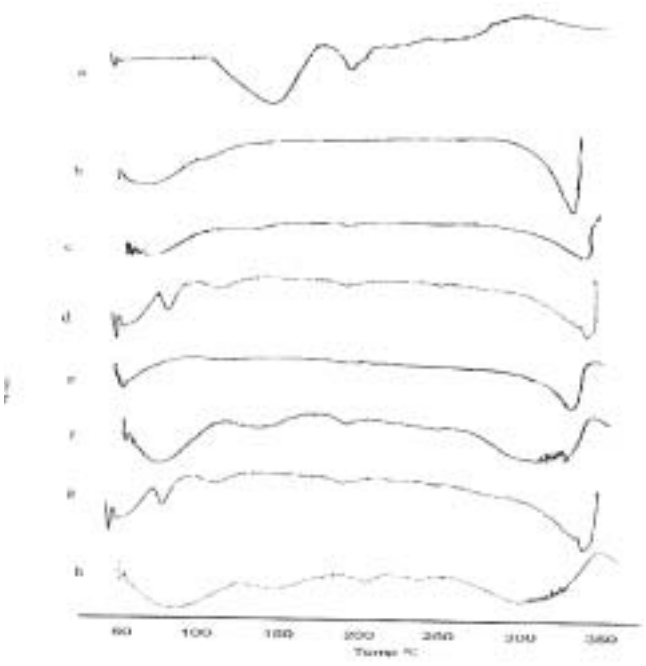

FIGURE 6 **DSC Thermogram of Rutin and Rutin-HP-ß-CD binary systems. (a) Rutin; (b)HP-ß-CD; (c) Rutin-HP-ß-CD 1:2 Kn; (d) Rutin-HP-ß-CD 1:1 Kn; (e) Rutin-HP-ß-CD 1:2 Co; (f) Rutin-HP-ß-CD 1:1 Co; (g) Rutin-HP-ß-CD 1:2 Pm; (h) Rutin-HP-ß-CD 1:1 Pm.**

showed similar shifts in peaks as mentioned above and as shown in Fig. 5. It is difficult to form an inclusion complex when both quercetin and ß-CD/HP-ß-CD are in the solid state. These results indicated the existence of interactions between quercetin and ß-CD/HP-ß-CD in prepared mixtures to form inclusion complexes.

The thermograms of rutin and its binary systems are shown in (Fig. 6) rutin-HP-ß-CD. The DSC thermogram of rutin exhibited a sharp endothermic peak at 143.62°C and 190.05°C indicating the melting point of rutin. For the kneaded mixtures (1:2 M) of rutin and ß-CD/HP-ß-CD, the drug endothermic peak shifted from 143.62°C to 118.4°C and the drug peak at 190.5°C shifted to 186.51°C. Similarly the ß-CD peak at 228.2°C shifted to 215°C. The DSC profile of coevaporated drug-ß-CD/HP-ß-CD mixtures and physical mixtures, showed similar shift in peaks as mentioned above Fig. 6.

Fourier Transform-Infra Red Spectroscopy

Infra red spectra of quercetin as well as those of its solid systems with CDs are presented in Fig. 7. Quercetin

FIGURE 7 **FT-IR Spectra of Quercetin and Quercetin-HP-ß-CD Binary Systems. (a) Quercetin; (b) HP-ß-CD; (c) Quercetin-HP-ß-CD 1:2 Kn; (d) Quercetin-HP-ß-CD 1:1 Kn; (e) Quercetin-HP-ß-CD 1:2 Co; (f) Quercetin-HP-ß-CD 1:1 Co; (g) Quercetin-HP-ß-CD 1:2 Pm; (h) Quercetin-HP-ß-CD 1:1 Pm.**

crystals show a characteristic carbonyl absorption band at 1664.4 cm^{-1}, assigned to aromatic ketonic carbonyl stretching. ß-CD and HP-ß-CD show characteristic absorption bands at 1636 cm^{-1} and 1640 cm^{-1} respectively. The FT-IR spectra of kneaded, coevaporated and physical mixtures were compared with those of ß-CD/HP -ß-CD, and pure drug. In the case of kneaded systems in particular, the characteristic aromatic carobonyl-stretching band of drug shifted to 1653.54 cm^{-1} and 1651 cm^{-1} for quercetin-ß-CD and quercetin-HP-ß-CD 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 (Pralhad & Rajendrakumar, 2004).

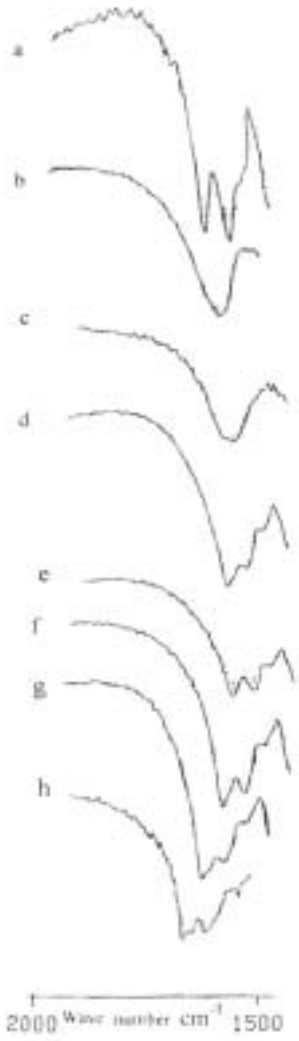

FIGURE 8 **FT-IR Spectra of Rutin and Rutin-HP-ß-CD Binary Systems: (a) Rutin; (b) HP-ß-CD; (c) Rutin-HP-ß-CD 1:2 Kn; (d) Rutin-HP-ß-CD 1:1 Kn; (e) Rutin-HP-ß-CD 1:2 Co; (f) Rutin-HP-ß-CD 1:1 Co; (g) Rutin-HP-ß-CD 1:2 Pm; (h) Rutin-HP-ß-CD 1:1 Pm.**

Infra red spectra of rutin as well as those of its solid systems with CDs are presented in Fig. 8. Rutin crystals show a characteristic carbonyl absorption band at 1657.4 cm^{-1} which is assigned to aromatic ketonic carbonyl stretching. In the case of kneaded systems in particular, the characteristic aromatic carobonyl-stretching band of drug shifted to 1653.5 cm^{-1} and 1641 cm^{-1} for rutin-ß-CD and rutin-HP-ß-CD complexes respectively, the spectra also showed reduced intensity of the same band.

Dissolution Studies

The dissolution characteristics of quercetin/rutin and cyclodextrin complexes are given in Table 2. More than 90% of drug was released within 10 min of the starting of dissolution when HP-ß-CD was the complexing agent. These values were much higher than those for physical mixtures as well as for pure drug. Further, the release rates from quercetin-ß-CD/rutin-ß-CD were lower than the corresponding values for HP-ß-CD mixtures (Figs. 9 and 10). Statistical analysis of the data by two way analysis of variance indicated significant difference within the release rates of drugs quercetin/rutin between HP-ß-CD/ß-CD complexes. Differences observed between methods are also significant. Knead-

TABLE 2A **Mean ± SD Values of DE_{30} for Quercetin and Quercetin-CD Binary Systems (n = 3)**

Product	DE_{30} (%)
Quercetin	9.5 ± 0.36
Quercetin-ß-CD 1:1 Kn	40.06 ± 0.231
Quercetin-ß-CD 1:2 Kn	49.48 ± 0.481
Quercetin-ß-CD 1:1 Co	37.83 ± 0.534
Quercetin-ß-CD 1:2 Co	46.12 ± 0.219
Quercetin-ß-CD 1:1 Pm	18.61 ± 0.305
Quercetin-ß-CD 1:2 Pm	25.45 ± 0.340
Quercetin-HP-ß-CD 1:1 Kn	79.46 ± 0.536
Quercetin-HP-ß-CD 1:2 Kn	83.86 ± 0.289
Quercetin-HP-ß-CD 1:1 Co	74.20 ± 0.167
Quercetin-HP-ß-CD 1:2 Co	82.42 ± 0.296
Quercetin-HP-ß-CD 1:1 Pm	40.77 ± 0.161
Quercetin-HP-ß-CD 1:2 Pm	47.01 ± 0.393

DE—Dissolution Efficiency at 30 min.
Kn—Kneading Method, Co—Coevaporation Method, Pm—Physical mixtures.

TABLE 2B **Mean ± SD Values of DE_{30} for Rutin and Rutin-CD Binary Systems (n = 3)**

Product	DE_{30} (%)
Rutin	15.43 ± 0.302
Rutin-ß-CD 1:1 Kn	60.13 ± 0.302
Rutin-ß-CD 1:2 Kn	69.43 ± 0.275
Rutin-ß-CD 1:1 Co	53.55 ± 0.308
Rutin-ß-CD 1:2 Co	61.22 ± 0.236
Rutin-ß-CD 1:1 Pm	31.33 ± 0.104
Rutin-ß-CD 1:2 Pm	35.76 ± 0.462
Rutin-HP-ß-CD 1:1 Kn	81.83 ± 0.305
Rutin-HP-ß-CD 1:2 Kn	86.80 ± 0.260
Rutin-HP-ß-CD 1:1 Co	75.62 ± 0.06
Rutin-HP-ß-CD 1:2 Co	84.94 ± 0.176
Rutin-HP-ß-CD 1:1 Pm	50.45 ± 0.265
Rutin-HP-ß-CD 1:2 Pm	60.91 ± 0.117

DE—Dissolution Efficiency at 30 min.
Kn—Kneading Method, Co—Coevaporation Method, Pm—Physical mixtures.

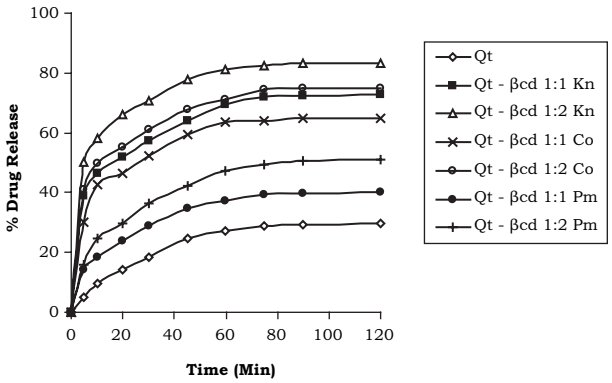

FIGURE 9A **Dissolution Profiles of Quercetin and Quercetin-ß-CD Binary Systems.**

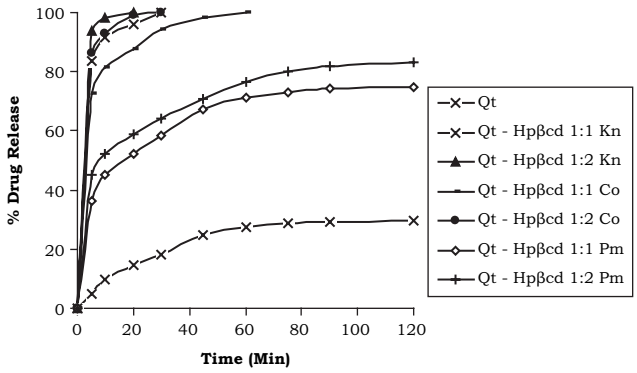

FIGURE 9B **Dissolution Profiles of Quercetin and Quercetin-HP-ß-CD Binary Systems, Qt - Quercetin, Kn - Kneading Method, Co - Coevaporation Method, Pm - Physical mixture.**

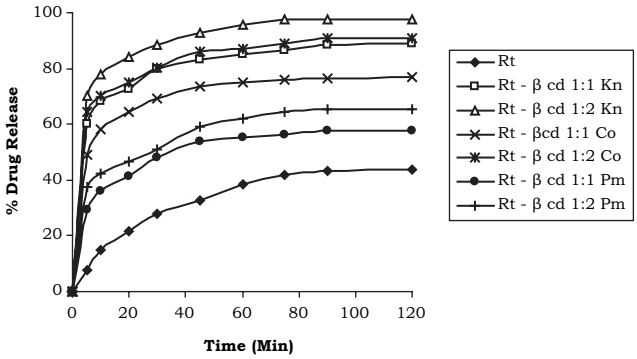

FIGURE 10A **Dissolution Profiles of Rutin and Rutin-ß-CD Binary Systems.**

ing method is superior to the other two methods. Dissolution efficiency at 30 min was calculated. The **DE_{30} (%) values** also support the above result. i.e., the kneaded mixtures of quercetin-HP-ß-CD/rutin-HP-ß-CD in 1:2 molar ratio showed higher dissolution efficiency (Table 2, Figs. 9 and 10) than the corresponding coevaporated and physical mixtures.

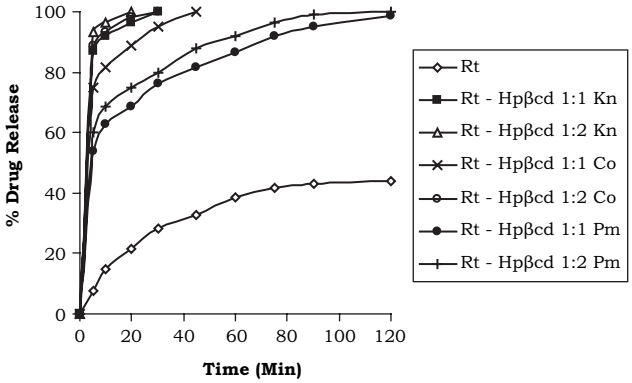

FIGURE 10B **Dissolution Profiles of Rutin and Rutin-HP-ß-CD Binary Systems, Rt - Rutin, Kn - Kneading Method, Co - Coevaporation Method, Pm - Physical mixtures.**

DISCUSSION & CONCLUSION

Cyclodextrins have attracted the attention of many formulation experts due to their nature of improving the solubility of poorly water soluble drugs. Among these CDs, β-CD and its hydrophilic derivative HP-ß-CD are preferred because of their suitable cavity sizes and low prices (Liu & Zhu, 2006). Hence, in our present work, CD inclusion complexes of quercetin/rutin were prepared. Analysis of the complexes by X-ray diffraction, DSC and FT-IR methods showed considerable interaction of β-CD and HP-ß-CD with quercetin/rutin. The observations are in agreement with those of the works reported earlier (Haiyun et al., 2003; Pralhad & Rajendrakumar, 2004; Calabro et al., 2005; Zheng et al., 2005).

Solid inclusion complexes prepared by kneading and coevaporation methods exhibited higher dissolution efficiencies than their corresponding physical mixtures. Improvement in the hydrophilicity of drugs by CDs might have contributed to the enhancement of dissolution rate. The kneaded complexes of quercetin/rutin with HP-ß-CD (1:2 molar ratio) were found to be superior to other complexes prepared. The better effectiveness of HP-ß-CD may be due to its greater water solubility, higher wetting and complexation ability.

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