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ORIGINAL ARTICLE

Enhancing the dissolution of hydrophobic guests using solid state inclusion complexation: characterization and in vitro evaluation

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Abstract The objectives of the present investigation were to prepare and characterize solid inclusion complexes of Etodolac (ETD) with β-cyclodextrin (β-CD) in order to study the effect of complexation on the dissolution rate of ETD, a hydrophobic guest molecule. Phase solubility curve was classified as a typical A_L-type for the cyclodextrins (CD's), showing that soluble complex was formed. The inclusion complexes in the molar ratio of 1:1 and 1:2 (β-CD–ETD) were prepared by various methods such as kneading, co-evaporation and in molar ratio of 1:1 by spray dried technique respectively. The molecular behaviors of ETD in all samples were characterized by nuclear magnetic resonance (NMR) spectroscopy, fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) studies and Scanning Electron microscopy (SEM) analysis. The results of these studies indicated that complexes prepared by kneading, co-evaporation and spray drying techniques showed inclusion of the ETD molecule into the CD's cavities. The highest improvement in in vitro dissolution profiles was observed in complexes prepared with spray dried technique. Mean in vitro dissolution time indicated significant difference between the release profiles of ETD from complexes and physical mixtures and from pure ETD.

Keywords ETD · β-Cyclodextrin · Kneading method · Co-evaporation · Spray drying technique

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Introduction

The poor dissolution characteristics of relatively insoluble drugs have been a problem to pharmaceutical industry. Retrospective studies show that >40% of drug failures in development can be traced to poor biopharmaceutical properties, especially poor dissolution or poor permeability [1]. The physicochemical properties of a drug mainly its hydrophilicity and lipophilicity has a major effect on its pharmacological activity. A drug when administered perorally needs to solubilized into the stomach. This solution is absorbed systemically through the biological membranes to act pharmacologically. To solubilize into the stomach, a drug must have some degree of aqueous solubility and to permeate biological membranes via passive diffusion, the drug must possess a similar lipophilicity. This hydrophilic–lipophilic balance of the drug is crucial for its overall pharmacodynamics [2].

There are a score of medicinally important drugs available, which are poorly soluble in aqueous fluids of gastrointestinal tract. Consequently, the dissolution rate of these compounds is low and their gastrointestinal absorption tends to be incomplete and erratic in other words they possess poor bioavailability. The required aqueous solubility of the drug is dependent upon its potency i.e. the dosage size and the type of formulation [3]. Etodolac (ETD) (±(RS)-1, 8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*] indole-acetic acid), is a pyrano-indole acetic acid derivative which acts as an anti-inflammatory, analgesic and antipyretic agent. The anti-inflammatory activity of ETD has been reported to be mainly due to the inhibition of prostaglandin biosynthesis [4]. ETD is indicated for acute pain and long-term use in the management of signs and symptoms of osteoarthritis and rheumatoid arthritis [5]. The recommended total daily dose of ETD for acute pain is up to 1000 mg, given as 200–400 mg

every 6–8 h. Major side effects include dyspepsia, abdominal pain, diarrhea, flatulence, nausea, constipation, gastritis, melena and vomiting etc. Other side effects include chills, fever, asthenia/malaise, dizziness, depression, nervousness, pruritus and rashes. This drug exhibit high poor solubility in water and this limits its use to solid dosage forms for oral administration [6]. Over the years a variety of techniques like pH adjustment, co-solvent addition, surfactant addition, etc. have been studied and widely used to improve the solubility and dissolution of water insoluble drugs. The formation of inclusion complex with nontoxic agents is also a type of manipulation used to improve the dissolution properties of drugs [7]. CD's, the cyclic oligomers of dextrose or its derivatives have been used extensively as such complexation agents. They increase drug solubility by forming an inclusion complex with the non polar region of the drug molecule (guest) being inserted into the cavity of the CD molecule (host) [8].

The objective of the present work was to prepare and characterize the drug–β-CD complexes and further to investigate the possibility of improving the solubility and dissolution of ETD by complexation with β-CDs.

Materials and methods

Materials

ETD was a generous gift from Ranbaxy Labs Pvt. Ltd. (Gurgaon, India). β-CD was obtained from S.A. Chemicals (Mumbai, India). All chemicals and solvents used in this study were of analytical grade. Fresh tripled distilled water was used throughout the work.

Phase solubility study

Solubility studies were carried out according to the method reported by Higuchi and Connors [9]. Excess amount of ETD (25 mg) was carefully weighed into a 50 ml conical flask to which 20 ml of aqueous solution containing β-CD at various concentrations (1.0×10^{-3} M–8.0×10^{-3} M) were added. The flasks were sealed and equilibrated by shaking at 25 °C and 37 °C. When equilibrium has reached (48 h), the samples were filtered through a 0.22 μm membrane filter (Sartorius cellulose nitrate filter, Germany). The filtrates were assayed for drug by ultraviolet spectroscopy (UV) at 252 nm. The apparent 1:1 stability constants, K_s, were calculated from the initial straight line portion of the phase solubility diagram according to the equation:

$$K_s = \text{Slope}/S_o(1 - \text{Slope})$$

where S_o is the saturation concentration of drug measured without β-CD.

Preparation of ETD–β-CD inclusion complexes

Complexes of β-CD with ETD were prepared in the molar ratio of 1:1 and 2:1 by different methods like physical mixture, co-evaporation, and kneading. For ease in discussion, the samples are designated with different abbreviations shown in Table 1.

By kneading method

ETD and β-CD in the molar ratio 1:1 (KD1) and 1:2 (KD2) was used to prepare the complexes by kneading method. β-CD was wetted with distilled water in a mortar until a paste was obtained. ETD was then added in divided proportions and the slurry was kneaded for about 1 h. Appropriate amount of water was added in order to maintain suitable consistency. The product obtained was washed with dichloromethane to remove the uncomplexed drug. Further, the product was then dried under vacuum at 40 °C for 48 h.

By co-evaporation method

ETD and β-CD were taken in the molar ratio 1:1 (CP1) and 1:2 (CP2). β-CD was dissolved in 15 ml of water. To this solution the drug was suspended. It was equilibrated for a time period of one week in a shaker water bath maintained at 37 °C. After equilibration, the solvent was evaporated at 40 °C. After evaporation the dried complex was scrapped. The product obtained was washed with dichloromethane to remove the uncomplexed drug. Further the product was then dried under vacuum at 40 °C for 48 h.

By spray-drying method

ETD and β-CD in the molar ratio 1:1 (SD) was dissolved in 100 ml of methanol and 100 ml of purified water respectively. The solutions were further mixed and spray dried (Jay Instruments Limited, Mumbai). The drying conditions were as follows: inlet temperature 80.3 °C; outlet

Table 1 Abbreviations used for different ratios of complexes formulated by various methods

S.No.	Method of preparation	Ratio (ETD:β-CD)	Sample code
1.	Kneading method	1:1	KD1
2.	Kneading method	1:2	KD2
3.	Co-evaporation	1:1	CP1
4.	Co-evaporation	1:2	CP2
5.	Spray dried method	1:1	SD1
6.	Physical mixture	1:1	PM1
7.	Physical mixture	1:2	PM2

temperature 54.2 °C; aspirator 65%; feed rate 12%; atomization air pressure 1.75 Kg/cm^2.

Physical mixtures

Physical mixtures of ETD with different ratio β-CD (1:1, PM1 and 1:2, PM2) were prepared by passing through sieve (#60) separately and then mixing both solids by simple blending.

Drug content

Accurately weighed 5 mg complex from each batch was dissolved in 20 ml of methanol/water (1:1) mixture. The drug content was assayed at wavelength of 278 nm by UV spectrophotometer (UV–Visible Spectrophotometer, Shimazdu-1601). Each determination was done in triplicate. Percent drug content was calculated for each sample by using the following formula:

$$\% \text{ Drug content} = \text{Measured drug in complex/Amount of complex taken} \times 100$$

NMR spectroscopy

^{1}H-NMR experiments were performed at 400 MHz using a Bruker AVANCE DPX 300 spectrometer. The probe temperature was regulated at 298 K. CD_3OD and D_2O (1:1 mixture) was used as solvent system in each case with tetramethyl silane as internal standard. The conditions for fourier transform measurements were as follows: acquisition time 5.19 s; pulse angle 30°; delay time 5 s; number of scans used 103. Shift value of the complexes (β-CD bound drug) and free β-CD are recorded in same condition and in same solvent system.

Differential scanning calorimetry

The thermal analysis of the complexes and physical mixtures were performed on Mettler Toledo® STAR System from 0° to 450 °C at 10 °C/min, the flow rate of nitrogen gas was kept at 20 ml/min.

Scanning electron microscopy

The surface characterization of inclusion complexes and physical mixtures were analyzed by SEM with much higher resolution under a scanning electron microscope (JSM 6100 JEOL, Japan). The samples were mounted onto stubs using double sided adhesive tape. The formulations were then coated with Au-Pd alloy (150–200 Å) using fine coat ion sputter (JEOL, fine coat ion sputter JFC-1100).

X-ray diffractometry

Powder X-ray diffraction patterns for all samples were obtained using X-ray diffractometer (Philips PW 1729 X-ray generator computer 1710) under the following conditions: target Cu; filter Ni; voltage 35 kV; current 20 mA; receiving slit 0.2 inches; x-axis 10 mm: 1° 2θ; y-axis 2000 cps using 'Ni' filtered Cu-k_α radiation as source.

FTIR spectroscopy

The FTIR spectra of the pure components, the inclusion complexes and the physical mixtures were taken on an IR spectrophotometer (60 MHz Varian EM 360 Perkin Elmer) using the KBr disk technique. The scanning range was 4500–400 cm^{-1}.

In vitro release profile studies

The dissolution studies were performed in USP dissolution apparatus number II (rotating paddle type). Accurately weighed complexes equivalent to 5 mg of ETD were spread over 900 ml of dissolution medium (phosphate buffer pH 7.2, official in BP). The stirring speed employed was 50 rpm and the temperature was maintained at 37 °C ± 0.5 °C. Ten milliliter aliquots of dissolution media were withdrawn at various time intervals and replaced by 10 ml of fresh dissolution media maintained at same temperature. The collected samples were analyzed spectrophotometrically. All the determinations were done in triplicate.

Results and discussion

Phase solubility studies

A typical A_L-type solubility curve (Fig. 1a, b) depicts that the solubility of ETD increased in a linear fashion as a function of β-CD concentration. The apparent stability constants of the inclusion complex (K_s) decreased with increasing temperature may be due to decrease in the interaction forces, such as van der waals and hydrophobic forces. The value for K_s was found to be 77 M^{-1} at 25 °C and 69 M^{-1} at 37 °C respectively. The changes in thermodynamic parameters during complexation may be attributed to changes in van der waals interaction energy, hydrogen bonding and hydrophobic interactions between guest molecule and CD. The enthalpy of the complexation reaction was −1957.14 J/mol at 25 °C. The negative sign indicated that the reaction was exothermic and associated with the release of energy that favored formation of the complex. The entropy change was observed to be

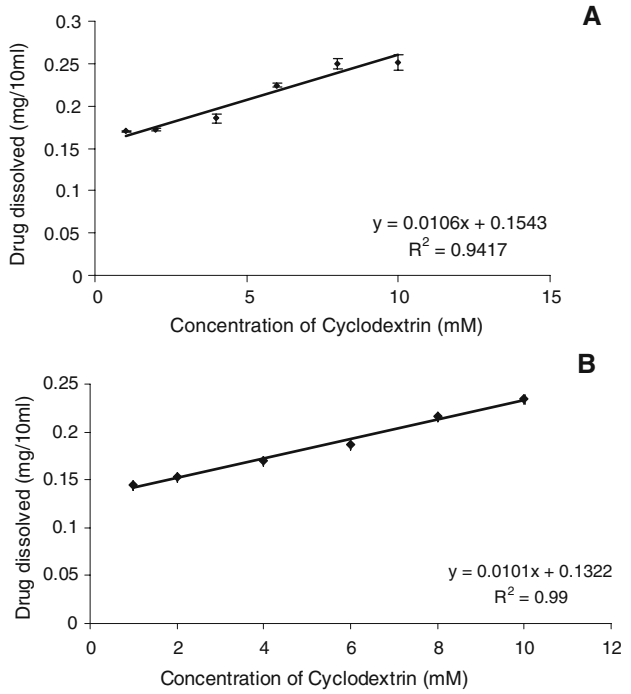

Fig. 1 Phase solubility diagram of ETD: β-CD in water at **a** 37 °C ($n = 3$) and **b** at 25 °C ($n = 3$)

−16.06 J/molK at 25 °C and −16.70 J/mol K at 37 °C. The negative sign indicated that complexation of ETD with β-CD resulted in increase in the order of the system.

^{1}H-NMR results

^{1}H-NMR spectroscopy was carried out for ETD/β-CD inclusion complexes in order to gain insight into the inclusion mode of the complex. Insertion of guest molecule into hydrophobic cavity of the host molecule resulted in the modification of NMR frequencies [10, 11]. The chemical shift values of NMR spectra of free ETD and free β-CD are depicted in Tables 2, 3. In case of physical mixture PM1 and PM2, there was no change in β-CD protons. The signals observed were sharp and distinct similar to free β-CD and free ETD spectra, which indicates no interaction between β-CD and ETD. Major changes in the chemical shift values were observed in the NMR of CD region (δ3.6–4.9), which gave information about the inclusion of guest molecule. Appreciable shift occurred in H-5 proton and H-3 proton in kneaded complexes KD1 and KD2 (Fig. 2a, b), which correlate the interaction of the drug and H-3/H-5 protons of β-CD [12].

A typical structural inference is that if, only H-3 proton undergoes a shift in the presence of substrate then the cavity penetration is shallow whereas if, H-5 proton undergoes change in shift position, then the penetration is deep [13]. The H-3 and H-5 protons located inside the cavity and the H-6 proton located on the cavity rim at the narrow end of the molecule have appreciably shifted. Therefore, in the present study it can be inferred that the ETD penetrates deeply into the β-CD cavity.

Table 2 Proton chemical shifts corresponding to ETD in absence and presence of β-CD ($\Delta\delta^* = \delta_{complex} - \delta_{etodolac(free)}$)

Proton	δ β-CD Free	$\delta_{1:1}$ (KD1)	$\Delta\delta^*_{1:1}$ (KD1)	$\delta_{1:2}$ (KD2)	$\Delta\delta^*_{1:2}$ (KD2)	$\delta_{1:1}$ (CP1)	$\Delta\delta^*_{1:1}$ (CP1)	$\delta_{1:2}$ (CP2)	$\Delta\delta^*_{1:2}$ (CP2)	ETD (SD)	$\Delta\delta^*$ ETD (SD)
H-3	6.9240	6.9027	−0.0213	6.9117	−0.0123	6.9148	−0.0094	6.9155	−0.0085	6.9307	0.0067
H-4	7.2578	7.2678	0.0100	7.2685	0.0107	7.2652	0.0074	7.2659	0.0081	7.2671	0.0093
H-5	6.9557	6.9398	−0.0159	6.9399	−0.0158	6.9428	−0.0129	6.9435δ	−0.0122	6.9540	−0.0017
H-11	2.0078	–	–	–	–	2.0203	0.0125	2.0245	0.0167	2.0189	0.0111
H-12	0.6368	0.6402	0.0034	0.6403	0.0035	0.6684	0.0316	0.6782	0.0414	0.6442	0.0074
H-13	2.7851	2.7354	−0.0497	2.7356	−0.0495	2.7895	−0.0044	2.7501	−0.0350	2.7900	0.0049
H-14	1.1971	1.2352	0.0381	1.2353	0.0382	1.2203	0.0232	1.2210	0.0239	1.2184	0.0213

Table 3 Proton chemical shifts corresponding to β-CD in absence and presence of ETD ($\Delta\delta^* = \delta_{complex} - \delta_{\beta\text{-CD(free)}}$)

Proton	δ ETD Free	$\delta_{1:1}$ (KD1)	$\Delta\delta^*_{1:1}$ (KD1)	$\delta_{1:2}$ (KD2)	$\Delta\delta^*_{1:2}$ (KD2)	$\delta_{1:1}$ (CP1)	$\Delta\delta^*_{1:1}$ (CP1)	$\delta_{1:2}$ (CP2)	$\Delta\delta^*_{1:2}$ (CP2)	ETD (SD)	$\Delta\delta$ ETD (SD)
H-1	4.9479	4.9563	0.0084	4.9564	0.0085	4.9129	−0.0350	4.9136	−0.0343	4.9159	−0.0320
H-2	3.5266	3.5315	0.0049	3.5319	0.0053	3.5241	−0.0025	3.5885	0.0619	3.5311	0.0045
H-3	3.8196	3.8280	0.0084	3.8281	0.0085	3.9952	0.1756	3.9959	0.1763	3.8196	0
H-4	3.4988	3.5039	0.0051	3.5040	0.0052	3.5073	0.0085	3.5080	0.0092	3.4955	−0.0033
H-5	3.6654	3.6741	0.0087	3.6742	0.0088	3.6501	−0.0153	3.6501	−0.0153	3.6741	0.0087
H-6	3.7842	3.7927	0.0085	3.7928	0.0086	3.7709	−0.0133	3.7716	−0.0126	3.7842	0

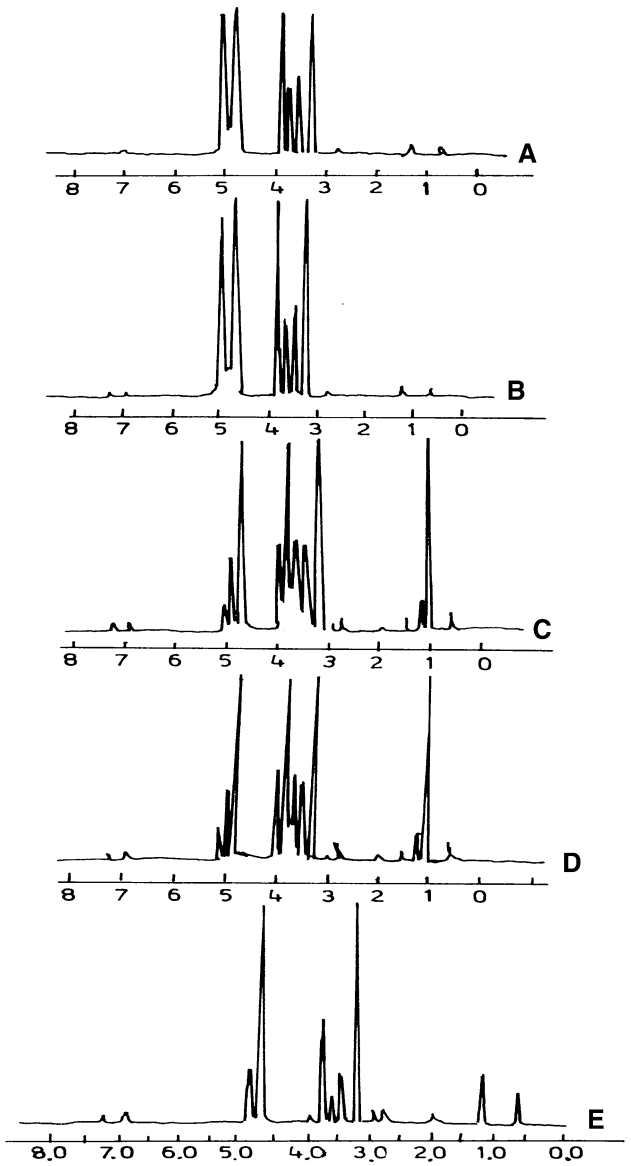

Fig. 2 NMR spectra of **a** KD1, **b** KD2, **c** CP1, **d** CP2, **e** SD

The downfield shifts in presence of β-CD were in range of 0.0049–0.0087 and 0.0052–0.0086 for kneaded complexes KD1 and KD2 respectively. The clear downfield shift of H-3 and H-5 proton has been attributed to presence of an electronegative group of guest molecule. In the ^{1}H-NMR spectra of KD1 and KD2 complexes all CD protons were deshielded indicating that the ETD molecule created magnetic anisotropic effects in the interior of the cavity due to weak interactions (van der waal's forces) with the internal hydrogen atoms [H-3 and H-5]. Similar observations were reported by many authors [14]. Change in the splitting pattern gives important indication of the inclusion complex formation. In case of free β-CD, H-5 proton (δ3.6654) was well resolved from H-6 and H-3 protons, which was very conspicuous, while it was introduced in the area of H-6 proton in case of inclusion complexes KD1, KD2 (Fig. 2a, b).

In case of kneaded complexes (KD1 and KD2), the ETD protons in complex form showed both downfield as well as upfield $\Delta\delta$ values in the range -0.0497 to 0.0381 for KD1 and -0.0495 to 0.0382 for KD2 (Table 2). The major changes were observed for all the ETD protons in complex form. The large downfield shift in H-4, H-12 and H-14 was due to the interaction of these protons with electronegative groups in the CD cavity. The upfield shift in H-3, H-5 and H-13 suggest that the aromatic part of ETD have been accommodated into the hydrophobic cavity of β-CD indicating that the ETD molecule created magnetic anisotropic effects in the interior of the cavity due to weak interactions (van der waal's forces) with the internal hydrogen atoms. The complexation pattern of KD1 and KD2 was similar and configuration of ETD in complexes was such that electronegative groups free methyl and H-3 aromatic protons of ETD were forming hydrogen bonds with oxygen of β-CD.

NMR spectra of co-evaporated complexes (as displayed in Fig. 2c, d) and the chemical shift value of CDs in the presence of ETD are depicted in Table 3. The shifts of CD for co-evaporated complexes were in range of -0.0350 to 0.1756 for CP1 and -0.0343 to 0.1763 for CP2. The major changes were observed in H-3 and H-5 protons of these complexes, which suggest deep interaction of guest molecule interior of the CD cavity. The large upfield shift of H-5 protons indicates that this proton was interacting with ETD by van der waal forces. The splitting patterns of CD protons had been changed significantly. In case of co-evaporated complexes (CP1 and CP2), the ETD protons in complex form showed both downfield as well as upfield $\Delta\delta$ values in the range of -0.0129 to 0.0232 for CP1 and -0.0350 to 0.0239 for CP2, the major changes were observed for H-5, H-3, H-13 protons which observed the upfield shift, which suggest that these protons are interacting with host H-3 and H-5 protons by long range forces. The downfield shift in H-14, H-12 and H-11 was due to interaction with electronegative groups of the CD cavity. The large shift in ETD protons in co-evaporated complexes indicate that ETD, interacted deep inside the cavity in which aromatic part of ETD was inside and complexation took place from hydrophobic region.

NMR spectra of spray-dried complex (Fig. 2e) and the chemical shift values of CDs in the presence of ETD are depicted in Table 3. In case of spray dried complex the shifts were in the range of -0.0320 to 0.0087 and the major changes were observed for H-1 and H-5 protons. The H-3 proton was not interacting which suggest that complex formation was shallow. The H-3 protons was not participating in the complex formation, which suggest the complex formation have taken place through the aromatic side

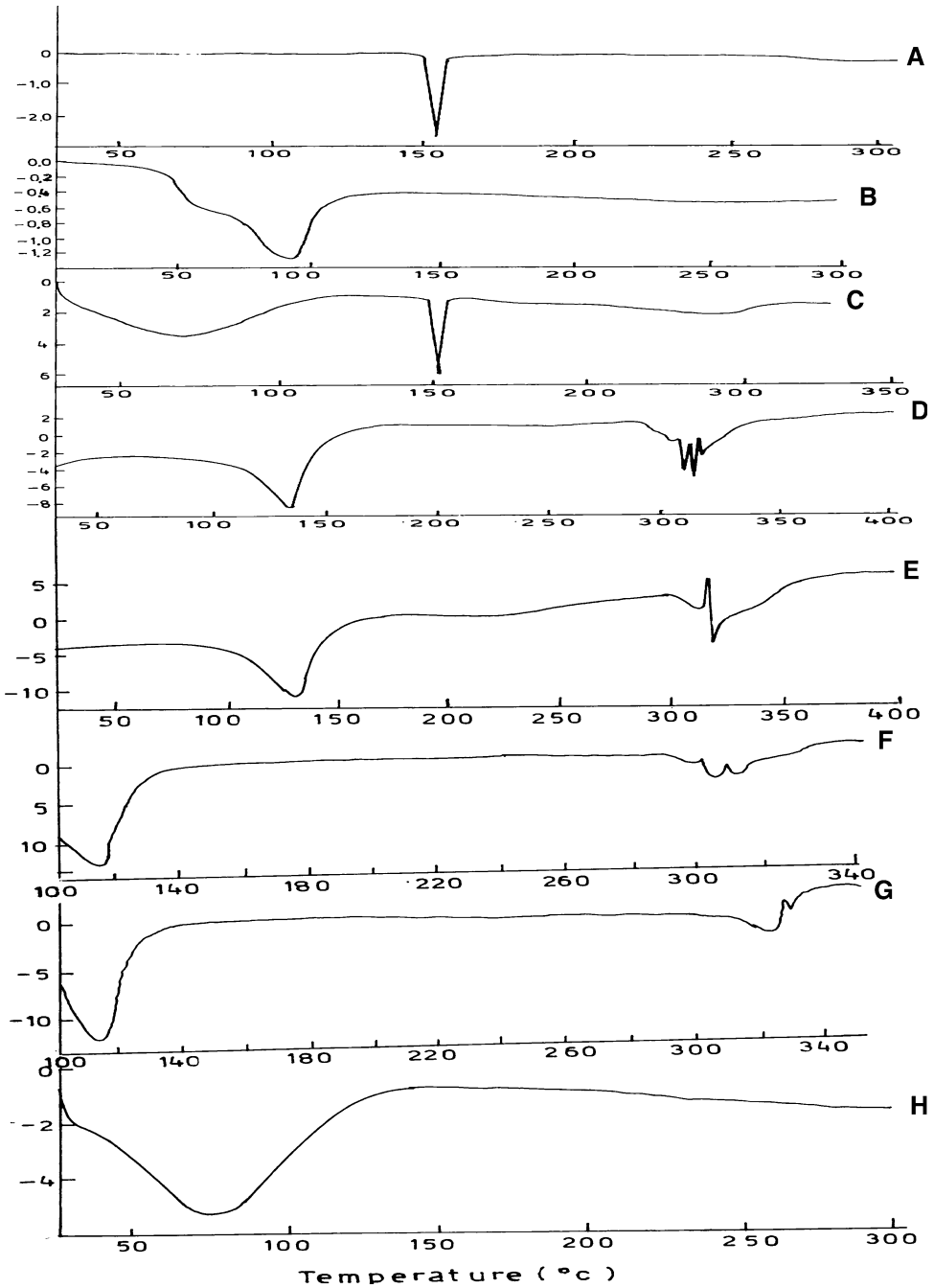

Fig. 3 DSC analysis of **a** Pure ETD, **b** β-CD, **c** PM1, **d** KD1, **e** KD2, **f** CP1, **g** CP2, **h** SD

of ETD in which conformation of ETD inside was different from co-evaporated and kneaded complexes.

In case of spray dried complex the shifts were in the range of −0.0017 to 0.0213 for ETD protons. The aromatic protons and hydrophobic region protons H-3, H-4, H-14 observed significant downfield shift. The H-5 proton was near electronegative group and H-14 is interacting by van der waal forces. The peaks of drugs were sharp and have multiple peaks, which suggest that some free drug was present on the surface of the complex. The complexation mode is by inclusion as well as adsorption.

In case of ETD and β-CD, good degree of complexation was observed. A significant downfield shift was observed for the protons of ETD in the presence of β-CD. Since under the condition employed only shift changes of the signal occurs. It follows that the complexation was a dynamic process in which a fast change exist between the free and bound state. The downfield signals of ETD and β-CD protons showed that guest was completely inside the cavity of the host. It was covered by the host through hydrogen bonding. The bonding pattern of kneaded and co-evaporated complexes was similar but bonding pattern

of spray-dried complex was different due to different ETD conformation. The splitting pattern of both ETD and β-CD were significantly altered when they were in complex form. The significant chemical shifts obtained for H-3 and H-5 protons were comparable to the results of inclusion obtained for barbiturates [15], cinnamic acid [16], where the aromatic moiety of guest molecule enters into the cavity of β-CD without any covalent bond formation. The same pattern in kneaded and co-evaporated complexes can be attributed to the linear structure of the guest.

DSC analysis results

DSC thermograms of pure ETD exhibited a sharp endothermic peak at 153 °C indicating the melting point of the drug as shown in Fig. 3a. Thermogram of β-CD showed a very broad endothermic peak in the range of 92.19 °C (Fig. 3b) due to elimination of water of crystallization as reported by many authors [17]. The endothermic peak of the drug was retained at 151.39 °C in physical mixture (Fig. 3c), while a broad peak corresponding to β-CD appeared in the range of 68.05 °C in case of physical mixture. These observations may be attributed to the presence of no interaction between the pure components in the physical mixture. DSC thermogram of KD1 and KD2 displayed an endothermic peak at 134.71 °C and 129.47 °C as displayed in Fig. 3d, e, respectively. The endothermic peak of ETD which appeared at 153 °C in case of the physical mixture was absent. This may be ascribed to the formation of inclusion complex. Similar results were observed by various authors [18, 19]. The solid complex obtained by co-evaporation method showed a broad endothermic in the range of 100–120 °C as shown in Fig. 3f, g. The free drug peak at 153 °C due to ETD was absent, suggesting an interaction between the drug and β-CD. The data suggested that complexation of drug in β-CD was achieved by the co-evaporation method and ETD might have formed an inclusion complex with β-CD. In case of spray dried complex (SD) (Fig. 3h) a broad endothermic peak in the range at 75.32 °C was observed which might be ascribed to the dehydration of the complexes. The endothermic peak at 150.63 °C due to ETD was present in case of SD which may be attributed to the presence of some uncomplexed drug in the complex [20, 21].

SEM analysis

The scanning electron microphotographs of ETD–β-CD inclusion complex and their physical mixture were obtained as depicted in Fig. 4. Pure ETD and β-CD exhibited typical crystalline surfaces as shown in Fig. 4a, b respectively. The physical mixture clearly depicted the crystalline structure of both ETD and β-CD. Inclusion complexes exhibited different characteristics of ETD and β-CD as compared to the one observed in physical mixture (Fig. 4c). In case of physical mixture, ETD appeared as irregular-shaped platy crystal habit and β-CD has presented somewhat parallelogram shape. The comparable morphology of these systems with pure components could reveal that apparently no ETD: β-CD interaction has taken place in the solid state. The corresponding inclusion complexes were constituted by relatively bulky particles (β-CD); with a surface morphology that was quite different from that observed in case of the physical mixture. A notable change in the shape and aspect of the β-CD was observed in the inclusion complexes.

The SEM of kneaded complexes were observed clearly (Fig. 4d, e) whereas the physical mixture showed the crystalline structure of ETD and β-CD, the features of both crystals in the kneaded complex were not easily detectable. Free drug crystals were also not observed. This observation suggested the existence of interaction between ETD and β-CD and formation of the inclusion complex of ETD with β-CD. The surface morphology of both the kneaded complexes (KD1 and KD2) was quite similar with no considerable difference.

However, in case of co-precipitated complex (Fig. 4f, g), the features of ETD crystals were not easily detectable. The surface morphology of the co-evaporated complexes was less amorphous as compared to the one observed in case of kneaded. No free drug particles were observed in the complex. Particles were present in the aggregated form. XRD of co-evaporated complex showed a reduction in crystallinity of the complex indicating interaction of ETD with β-CD. In the spray dried products the original morphology of ETD and β-CD disappeared and it was not possible to differentiate the two components. Spray dried system (SD) showed amorphous and homogenous aggregates of spherical particles as shown in Fig. 5h. Holes were observed on the surface of few particles suggesting the formation of few hollow spheres. The drastic change in the surface morphology of the spray-dried complexes was indicative of the presence of a new solid phase, which may be due to the molecular encapsulation of drug in the β-CD. XRD patterns of SD also indicated formation of amorphous complex. This indicated the presence of strong interaction between the drug and β-CD.

X-ray diffractometry

The powder X-ray diffraction patterns of pure ETD, β-CD and their physical mixtures and inclusion complexes were obtained as depicted in Fig. 5. The diffractogram of ETD exhibited a series of intense peaks which were indicative of their crystallinity (Fig. 5a). β-CD also exhibited many characteristic peaks due to its crystalline nature (Fig. 5b).

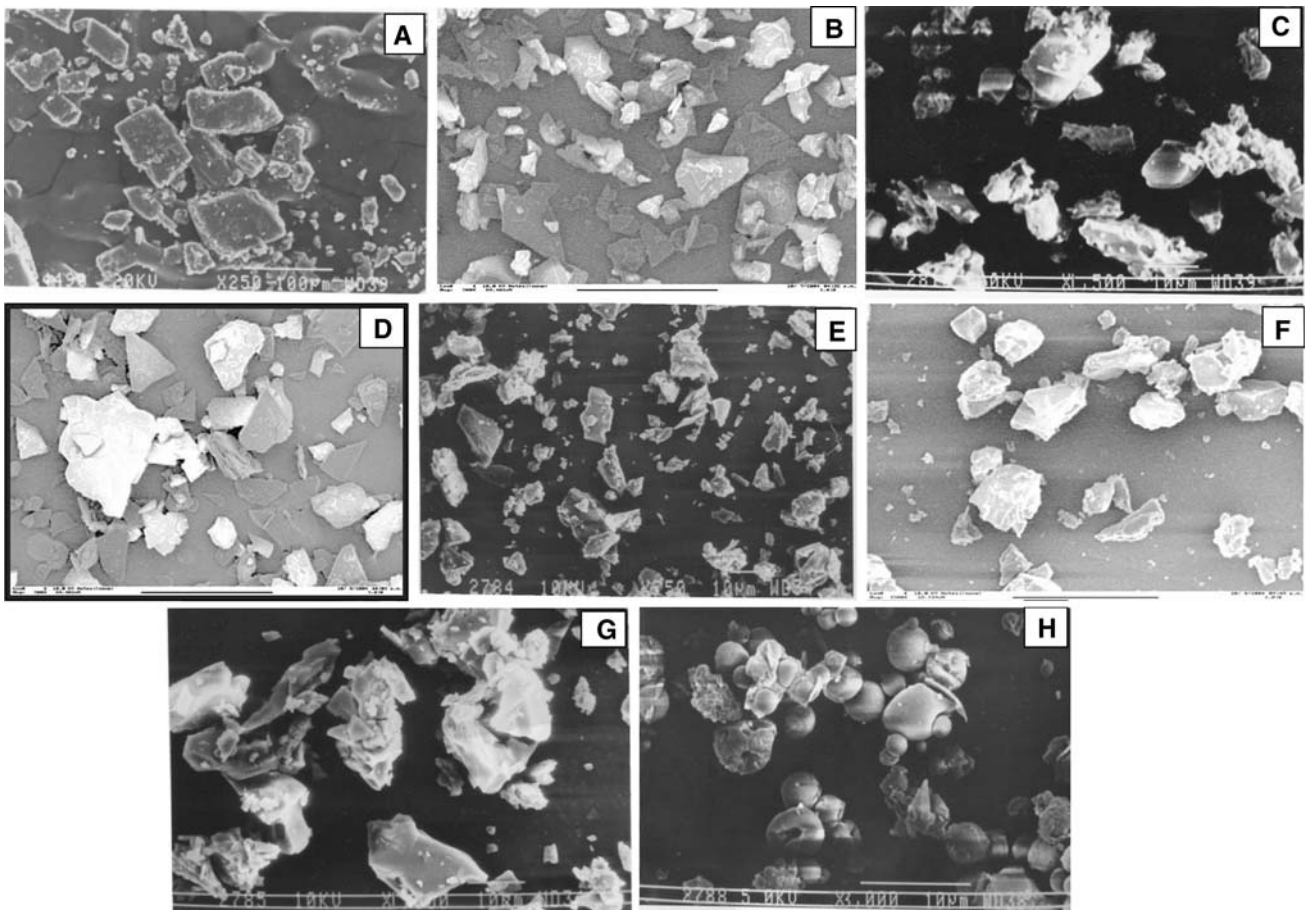

Fig. 4 SEM analysis of **a** β-CD, **b** ETD, **c** PM1, **d** KD1, **e** KD2, **f** CP1, **g** CP2, **h** SD

Most of the principal peaks of ETD and β-CD were present in the diffraction patterns of physical mixtures (Fig. 5c). This indicated that there was very less interaction between the drug and β-CD in case of physical mixtures. In contrast to the above observation, kneaded complexes of ETD and β-CD (KD1 and KD2, Fig. 5d, e) showed disappearance of major peaks corresponding to both ETD and β-CD. Many peaks of pure ETD were not present but several new peaks were also observed in the XRD of complex which may be indicative of formation of complex as reported by Sinha et al. [22]. The peaks observed were indicative of crystallinity of the drug.

However, in co-evaporated complexes of ETD and β-CD (CP1 and CP2, Fig. 5f, g) showed disappearance of major peaks corresponding to both ETD and β-CD. Some of the characteristic peaks of ETD were missing in the diffraction pattern of the co-evaporated complexes. Many peaks of pure ETD were not present but several new peaks were also observed in the XRD of complex which may be indicative of formation of complex. The above observation may be attributed to an interaction between ETD and β-CD showing the presence of a new solid state where a possible formation of an inclusion complex was contemplated. These results are in concordance with SEM studies.

Furthermore, the spray dried complexes of ETD and β-CD (SD) showed an altogether different diffraction pattern with the disappearance of all the peaks corresponding to both ETD and β-CD (Fig. 5h). The observed diffraction pattern was completely different from the ones obtained for the pure components as well as the physical mixtures. Very few peaks were observed and the peaks were different from the one observed in the case of pure drug as well as pure β-CD. The peaks observed were also not very sharp as observed in case of the other complexes. This was indicative of the transformation of ETD from the crystalline to the amorphous state by formation of an inclusion complex with β-CD using the spray drying technique.

IR spectroscopy

The interaction between host and guest molecule was effectively characterized by IR spectroscopy. IR spectrum of ETD showed medium absorption bands at 1740 cm^{-1} due to –COOH group and 3580 cm^{-1} that were assigned to

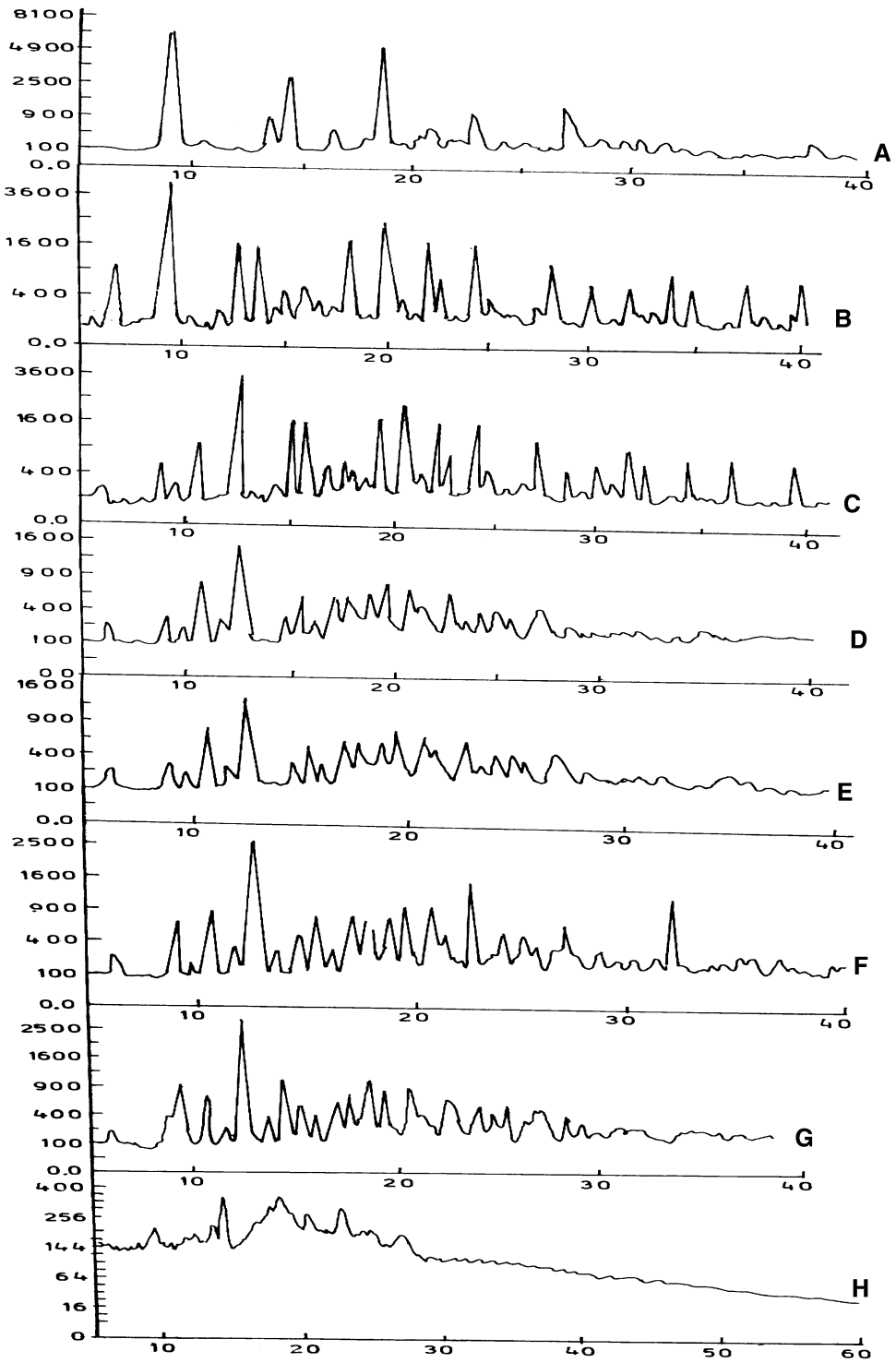

Fig. 5 PXRD analysis **a** Pure ETD, **b** β-CD, **c** PM1, **d** KD1, **e** KD2, **f** CP1, **g** CP2, **h** SD

drug –NH symmetric and asymmetric stretching vibrations, respectively. The other characteristic band at 1420 cm^{-1} may be attributed to the C–O–C symmetric and asymmetric stretching vibrations (Fig. 6a).

IR spectrum of β-CD showed a broad absorption band at 3372.2 cm^{-1} due to –OH stretching (Fig. 6b). However, physical mixtures in both ratios retained the characteristic –NH absorption band of ETD at 3343.8 cm^{-1} and 3344.3 cm^{-1} respectively, Also there was no shift in the C=O stretching vibration of ETD at 1740 cm^{-1} which appeared at 1745.0 cm^{-1} and 1745.2 cm^{-1} (Fig. 6c). These observations led to the conclusion that there was less

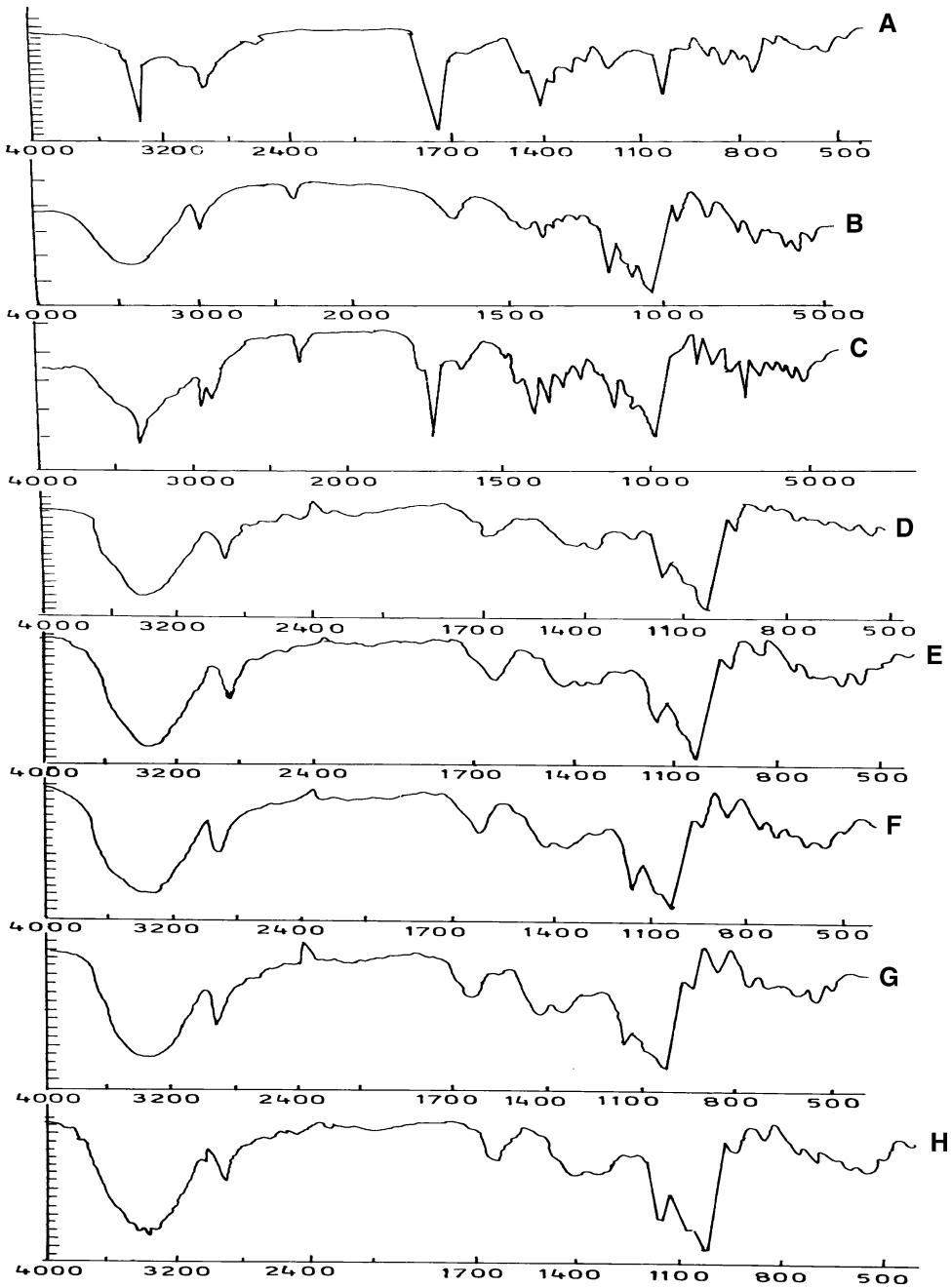

Fig. 6 FTIR spectra of **a** Pure ETD, **b** β-CD, **c** PM1, **d** KD1, **e** KD2, **f** CP1, **g** CP2, **h** SD

interaction between ETD and β-CD in the physical mixture. The –NH stretching bands of ETD at 3580 cm^{-1}were masked in the complexes.

In case of Kneaded complexes, the characteristic –NH stretching band symmetric at 3580 cm^{-1} were shifted in both KD1 and KD2. It appeared as a single broad peak at 3392.6 cm^{-1} in case of KD1 and at 3395.5 cm^{-1} in case of KD2 (Fig. 6d, e). This suggests breakdown of intermolecular hydrogen bonds of the crystals and formation of a monomeric dispersion of a drug as a consequence of the interaction with β-CD which could result in inclusion of the drug in the hydrophobic cavity. The C=O band at 1740.0 cm^{-1} was missing in the kneaded complexes. The broadening of -NH stretching vibration, and masking of C=O bands in kneaded complexes signify the existence of some interaction between the drug and β-CD.

However, in co-evaporated complexes, the characteristic NH stretching band symmetric at 3580 cm^{-1} was shifted in CP1 and CP2 (Fig. 6f, g). It appeared at 3392.8 cm^{-1} in case of CP1 and 3345.1 cm^{-1} in case of CP2. The C=O stretching at 1740.0 cm^{-1} was missing in case of the co-evaporated complexes. The broadening of –NH stretching vibration, and masking of C=O bands in kneaded complexes suggest some interaction between the drug and β-CD.

In spray dried complex, the characteristic –NH stretching band symmetric at 3580 cm^{-1} were shifted in the spray dried complex and appeared as a single broad peak at 3380.0 cm^{-1} (Fig. 6h). The C=O band at 1740.0 cm^{-1} was modified in the spray dried complex. It appeared as a short peak at 1700.0 cm^{-1}. This shortening of the peak may suggest that there can be minor quantities of the free drug present in the complex. The broadening of -NH stretching vibration, and shortening of C=O bands in spray dried complex signify the existence of some interaction between the drug and β-CD.

In vitro dissolution studies

In the case of pure drug, only 44.86% of the drug was dissolved even after 30 min as shown in Fig. 7. The dissolution of ETD from the powder alone was incomplete even after 90 min (82.35%). However, the dissolution profile of PM1 and PM2 showed slightly higher amount of drug dissolved at each sampling interval as compared with dissolution profile of pure drug.

An enhanced rate and extent of drug release was obtained at each time intervals in case of KD1 and KD2 over pure drug and physical mixtures (Fig. 7a). KD1 released 93.50% of the drug in 30 min while KD2 showed about 98.79% drug release in same time. Complete drug release was observed from kneaded complexes KD1 and KD2 in 90 min. Batch KD2 demonstrated slightly higher enhancement of dissolution compared to KD1 as depicted in Fig. 7a.

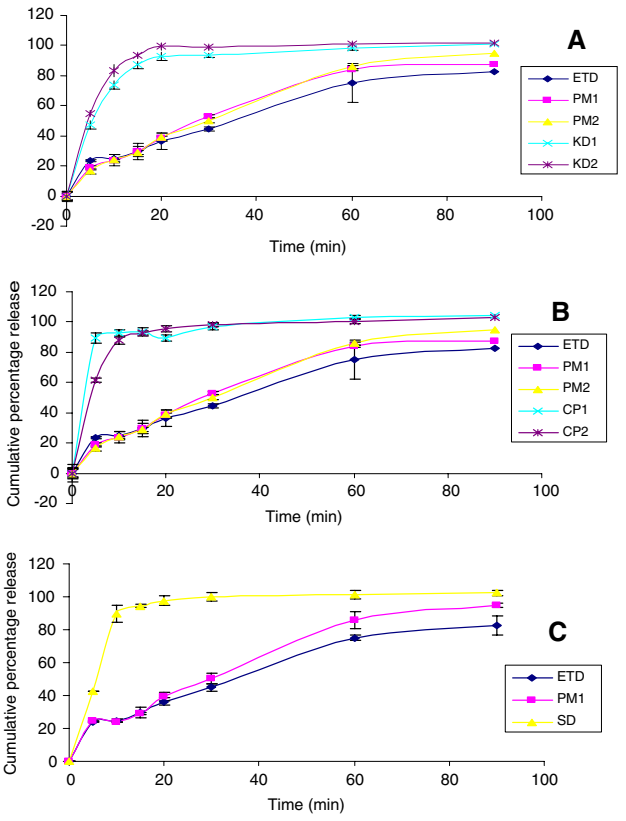

Fig. 7 Comparative in-vitro dissolution profiles of inclusion complexes in different ratios with respect to pure ETD. **a** Kneading complexes KD1 and KD2, **b** Co-evaporation complexes CP1 and CP2, **c** Spray-dried complexes SD

The significant improvement in dissolution characteristics of the complexes was attributed to reduced interfacial tension between the solid particles of ETD and the dissolution medium, leading to greater rate of dissolution. The increase in the dissolution rate of ETD physically mixed with β-CD was possibly due to local solubilization operating in the micro environment or the hydrodynamic layer surrounding the drug particles as reported by Becirevic-Lacan et al. [23]. In situ inclusion process might have resulted in increased amount of dissolved drug in case of physical mixtures [24].

The $t_{50\%}$ of kneaded complexes, KD1 and KD2 was 6 and 4 min respectively. The values of $t_{50\%}$ of both kneaded complexes were much faster than pure ETD ($t_{50\%} >$ 30 min.). The mean percent release of drug from kneaded complexes KD1 and KD2 at 30 min. was 2.08 and 2.20 fold higher with respect to pure ETD. The physical mixtures PM1 and PM2 also increased the mean percent release of drug by 1.18 and 1.12 fold respectively.

A higher rate and extent of drug release was obtained at each time intervals in case of CP1 and CP2 over pure drug and physical mixtures as depicted in Fig. 7b. Batch CP1 released about 92.72% of drug in 10 min whereas CP2 released 88.01% at 10 min. However, CP1 released 98.24% of the drug in 30 min while CP2 showed about 98.12% drug release in same time. Complete drug release was observed from both the complexes after 90 min of dissolution. It can be observed from Fig. 7b that CP1 demonstrated higher enhancement of dissolution at initial hours compared to CP2.

The $t_{50\%}$ of co-evaporated complexes, CP1 and CP2 was 3 min. and 4 min. respectively. The values of $t_{50\%}$ of both co-evaporated complexes were much faster than ETD alone ($t_{50\%} >$ 30 min). The mean percent release of drug from co-evaporated complexes CP1 and CP2 at 30 min was 1.99 and 2.18 fold higher with respect to pure ETD. The physical mixtures PM1 and PM2 also increased the mean percent release of drug by 1.18 and 1.12 fold respectively at 30 min. These data suggest that the co-evaporated complexes are more effective in increasing the dissolution rate than the physical mixtures. The mean percent release of ETD at 30 min was 98.24, 98.12 and 44.86% for CP1, CP2 and pure ETD respectively while for physical mixture it was 53.01 and 50.27% respectively.

Similarly, an enhanced rate and extent of drug release was obtained at each time intervals in case of SD over pure

drug and physical mixtures (Fig. 7c). Drug released from the SD was 99.96% in 30 min. Complete drug release was observed from the spray dried complex after 30 min of dissolution. The $t_{50\%}$ of SD was 7 min. The mean percent release of drug from SD at 30 min was 2.23 fold higher with respect to pure ETD.

Conclusion

The enhanced rate and extent of drug release was found to be higher among all the complexes as compared to pure drug. Kneaded (2.08 and 2.20 fold increase) and spray dried complexes (2.23 fold increase) produced higher dissolution rate than that of co-evaporated complex (1.99 and 2.18 fold) w.r.t pure ETD. Kneading, co-evaporation and spray drying techniques were appropriate to achieve complexation for hydrophobic guests like ETD, as confirmed by NMR, FTIR, PXRD, DSC and SEM studies in the solid complexes when compared to the corresponding physical mixtures revealing the formation of true inclusion complex. These findings proved greater utility in the fast dissolving dosage forms with possible enhancement of oral bioavailability for poorly dissolved drugs.

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