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Inter-molecular physiochemical characterization for etodolac-hydroxypropyl-β-cyclodextrin polymeric systems in solid and liquid state

Research Article

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Abstract: The purpose of this study was to explore the utility of hydroxypropyl-β-cyclodextrin (HP-β-CD) systems in forming inclusion complexes with the anti-rheumatic or anti-arthritic drug, etodolac (EDC), in order to overcome the limitation of its poor aqueous solubility. This inclusion system achieved high solubility for the hydrophobic molecule. The physical and chemical properties of each inclusion compound were investigated. Complexes of EDC with HP-β-CD were obtained using the kneading and co-evaporation techniques. Solid state characterization of the products was carried out using Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), powder X-ray diffraction (XRD) and Scanning electron microscopy (SEM). Studies in the solution state were performed using UV–Vis spectrophotometry and ^{1}H-NMR spectroscopy. Phase solubility profiles with HP-β-CD employed was found to be A_L type. Stability constants (K_c) from the phase solubility diagrams were calculated indicating the formation of 1:1 inclusion complex. Stability studies in the solid state and in liquid state were performed; the possible degradation by RP-HPLC was monitored. The dissolution studies revealed that EDC dissolution rate was improved by the formation of inclusion complexes.

Keywords: *Etodolac (EDC) • Hydroxypropyl-β-cyclodextrin (HP-β-CD) • Inclusion-complexes • Stability • Dissolution*

1. Introduction

Drugs can be modified by complexation to improve physicochemical properties such as dissolution. Lead compounds offered by high-throughput drug discovery methodologies are mainly difficult type II compounds. Complexation using cyclodextrins can be an important enabling technology for these compounds in particular. They help by increasing the apparent water solubility of a drug candidate, thereby developing formulations in which a type II molecule behaves like a type I, with an increase in oral bioavailability [1].

Cyclodextrins (CDs) have been used extensively in pharmaceuticals to form such complexes. Cyclodextrins encircle the drug molecules in the hydrophobic cavity formed by several molecules of cyclodextrin, with no covalent bonding. Formation of the inclusion complex involves molecular encapsulation of the guest molecule by cyclodextrin resulting in modification of such physicochemical properties as solubility and stability of the guest molecule [2-4]. Cyclodextrins are able to enhance the aqueous solubility of drugs without affecting their intrinsic ability to permeate biological membranes. The behavior of cyclodextrins in these complexes is affected by steric factors, proximity of charge to the cyclodextrin cavity, charge density, charge state of the cyclodextrins, nature of the drug, temperature, and co-solvent effects [5].

NSAIDs have now become the most widely prescribed and used drugs in the community for rheumatologic as well as non-rheumatologic conditions which include acute and chronic pain, biliary uretric colic, dysmenorrheal fever, closure of patent ductus arteiolus in infants. Nearly $2 billion is spent yearly in the United States on filling prescriptions of NSAIDs alone [6].

Recently various studies involving inclusion complexation phenomena have been reported for new generation NSAIDs which are almost insoluble in water, primarily to enhance the oral bioavailability and dissolution behavior [7-9]. EDC((*RS*)-2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b]indol-1-yl)acetic acid) is a newer NSAID belonging to the arylalkanoic class, which

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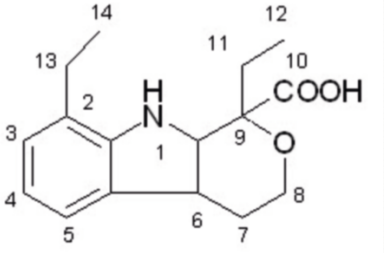
has been developed for the treatment of rheumatoid arthritis and osteoarthritis. It has superior in-vitro and in-vivo inhibitory action against the inducible isoform of COX-1 inhibition, which is associated with gastric, renal and other adverse effects. In animal models it has anti-inflammatory effects similar to or better than those of other NSAIDs, and a greater therapeutic ratio (exhibit low ulcerogenic potential with high efficacy) [10]. EDC is completely absorbed from the gastrointestinal tract with peak plasma concentrations occurring about 1-2 hours after ingestion. EDC is more than 99% bound to plasma proteins and has an elimination half life of 7.3 hours. The major drawback associated with the drug is the poor solubility in oral dosage forms.



Scheme 1. Structure of EDC.

The objective of the present study is to formulate inclusion complexes of EDC with different ratios of HP-β-CD using various preparation methods like kneading and co-evaporation. Intermolecular complexes formed were evaluated in solution using solubility studies, UV spectroscopy and proton magnetic resonance spectroscopy and in the solid state using X–ray diffraction, DSC and IR spectroscopy. Dissolution studies were performed for all inclusion complexes, and the overall results were compared with those obtained for the pure drug and for simple physical mixtures of drug and cyclodextrin.

2. Experimental Procedure

EDC (±, RS-1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b] indol-1-yl)acetic acid) was a generous gift sample provided by Ranbaxy Pvt Ltd., (Gurgaon, India) and HP-β-CD was obtained from S.A Chemicals (Mumbai, India). Freshly prepared triple distilled water was used throughout the study. All the chemicals and solvents used were of analytical grade.

2.1. Phase Solubility Studies

About 25 mg of EDC((*RS*)-2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b]indol-1-yl)acetic acid) was carefully weighed into a 50 mL conical flask to which 20 mL of aqueous solution containing HP-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 [11]. 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 UV Spectroscopy (UV Spectrophotometer, Shimadzu-1601) at 252 nm. The apparent 1:1 stability constants, K_c, were calculated from the given equation:

$$K_C = \frac{Slope}{S_0(1 - Slope)}$$

2.2. Preparation of Physical Mixtures

Physical mixtures of EDC and different ratios of HP-β–CD were prepared by passing the drug and HP-β–CD through (mesh#60) separately and then mixing both solids by simple blending in molar ratios 1:1 and 1:2 for EDC: HP-β–CD respectively.

2.3. Preparation of EDC–HP-β–CD Inclusion Complexes

Various techniques used for the preparation of inclusion complexes are discussed below. For ease in discussion, the samples are designated with different abbreviations as shown in Table 1.

2.3.1. Kneading Method

EDC and HP-β–CD in the molar ratios 1:1 (KDHP1) and 1:2 (KDHP2) were used to prepare the complexes by the kneading method. CD was wetted with water in a mortar until a translucent paste was obtained. EDC was then added in several portions and the slurry was kneaded for about 1 h. During the additions appropriate amounts of water were added in order to maintain a suitable consistency. The product obtained was washed with dichloromethane to remove the uncomplexed drug. The product was then dried under vacuum at 40˚C for 48 h.

2.3.2. Co-evaporation Method

EDC and HP-β–CD were taken in the molar ratios 1:1 (CPHP1) and 1:2 (CPHP2). β-CD was dissolved in 15 mL

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

Method of preparation	Ratio (EDC:HP-β-CD)	Sample code
Kneading	1:1	KDHP1
Kneading	1:2	KDHP2
Co-evaporation	1:1	CPHP1
Co-evaporation	1:2	CPHP2
Physical mixture	1:1	HPPM1
Physical mixture	1:2	HPPM2

of water. The drug was suspended and equilibrated in each solution 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. The dried complex was scraped. The product obtained was washed with dichloromethane to remove the uncomplexed drug. The product was then dried under vacuum at 40°C for 48 h.

2.4. Drug Content

5 mg of complex from each batch was accurately weighed and then dissolved in 20 mL of methanol/water (1:1) mixture. The drug content was spectrophotometrically assayed at 278 nm by UV Spectrophotometer (Shimadzu-1601, USA). Each determination was done in triplicate. Percent drug content was calculated for each sample by using the following formula:

$$\% \text{ Drug Content} = \frac{\text{Measured drug amount in complex}}{\text{Amount of complex taken}} \times 100$$

2.5. Physicochemical Characterization of Solid Complexes and Physical Mixtures

2.5.1. NMR Spectroscopy

^{1}H NMR experiments were performed at 500 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 (TMS) as internal standard. The conditions for fourier transform measurements were as follows: acquisition time 5.19 sec; pulse angle 30°; delay time 5 sec; number of scans used 103. Shift values for the complexes (HP-β–CD bound drug) and free HP-β–CD are recorded in the same conditions and in the same solvent system.

2.5.2. Differential Scanning Calorimetry

DSC of the complexes and physical mixtures were performed on Mettler Toledo STAR System. Samples were placed in sealed aluminum pans before heating under nitrogen flow (20 mL min^{-1}) at a heating rate of 10°C min^{-1} from 0°C to 450°C. An empty aluminum pan was used as reference. The equipment was periodically calibrated with Indium.

2.5.3. Scanning Electron Microscopy

The external morphology of inclusion complexes and physical mixtures were analyzed by 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 (150–200Å) using fine coat ion sputter (JEOL, fine coat ion sputter JFC–1100).

2.5.4. 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–Kα radiation as source.

2.5.5. 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 cm^{-1} – 400 cm^{-1}.

2.5.6. In-vitro Release Profile Studies

Accurately weighed complex samples (equivalent to 5 mg of EDC) were spread over 900 mL of dissolution medium (phosphate buffer pH 7.2, official in BP) in USP dissolution apparatus II (rotating paddle type). 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 with 10 mL of fresh dissolution medium maintained at the same temperature. The samples collected were analyzed spectrophotometrically at 278 nm by UV Spectrophotometer (Shimadzu-1601, USA). All the determinations were done in triplicate.

2.5.7. HPLC Analysis

Samples were injected manually using Rheodyne injector (Rheodyne, USA) with 20 µl loop to a Shimadzu quaternary gradient LC-10 AT VP HPLC equipped with a FCV-10 AL VP pump, DGU 14A degasser, SCL-10 AVP system controller, CTO-10AS column and SPD 10A VP UV-VIS detector. For chromatography, a C-18 reverse phase column (YMC HPLC column, YMC Co. Ltd. Japan, 250×4.6 mm, S-5µ) operated at ambient temperature was used. The wavelength of detection was set at 225 nm. The mobile phase used was Acetonitrile: buffer (pH 4.75) in the ratio of 45:55 at flow rate of 1 mL min^{-1}.

2.5.8. Stability Studies

To assess the stability of the EDC complexes, following studies were performed for 3 months. The formulations were tested periodically for changes in physical appearance, drug content, and drug release characteristics. For stability studies, the methods used were:

(a) *Thermal methods - use of Differential Scanning Calorimetry (DSC)*

Samples were analyzed by DSC for the determination of drug characteristics like melting peak or degradation peak at respective endotherms to indicate the purity or degradation of drug in complexes.

(b) *Quantitative assay after an isothermal stress test.*

In this test, inclusion complexes and comparison controls were subjected to stress conditions at 40°C/75% RH for 3 months. The samples were quantitatively analyzed for their drug content using RP-HPLC.

3. Results and Discussion

3.1. Phase Solubility studies

Phase solubility diagrams (as depicted in Fig. 1) showed a typical A_L-type solubility curve, indicating that the solubility of EDC increased in a linear fashion as a function of HP-β–CD concentration. The apparent stability constants of the inclusion complex (K_s) decreased with increasing temperature, probably due to decrease in the interaction forces, such as van der Waals and hydrophobic forces. The enthalpy was found to be -5063.47 J mol^{-1} at 25°C, indicating that the complexation is an exothermic reaction. The release of energy indicates that complex formation is favored. Also, the entropy change was negative (-5.23 J mol^{-1} K at 25°C and 37°C) which indicated that complexation increased the order of the system.

The intermolecular insertion of EDC into the hydrophobic HP-β-CD cavity resulted in modification of NMR shifts. In case of the physical mixtures, HPPM1 and HPPM2, there was no change in HP-β-CD and EDC proton signals (Figs. 2A and 2B). The signals obtained were sharp and distinct, similar to free HP-β-CD and EDC spectra which indicated less interaction. The splitting pattern of EDC and HP-β-CD did not change in HPPM1 and HPPM2.

3.2. NMR Spectral Studies

NMR spectra of kneaded complexes (KDHP1 and KDHP2, Figs. 2C and 2D) and co-evaporated complexes (CPHP1 and CPHP2, Figs. 2E and 2F) show major changes in the chemical shift values observed in the HP-β-CD region (δ3.5–5.0). Some comparative chemical shifts of HP-β-CD and of EDC in the complexes are listed in Table 2 and Table 3 respectively.

The downfield as well as upfield shifts for kneaded complexes and co-evaporated complexes signify the groups involved in complex formation (in range of 0.0067 to 0.0485 for KDHP1 and -0.0078 to 0.0407 for KDHP2) due to H-1, H-3, H-4 and H-5 protons and (in range of 0.0009 to 0.0514 for CPHP1 and -0.0012 to 0.0407 for CPHP2) due to H-1, H-4 and H-5 protons respectively. Although multiplicity of the HP-β-CD signals remained constant in case of KDHP1 and KDHP2, the protons of the HP-β-CD cavity undergo greater EDC induced chemical shift changes than those on the exterior of the torus. As the shift was observed both for H-3 and H-5 protons, it can be concluded that EDC penetrates deep and interacts inside the cavity [12].

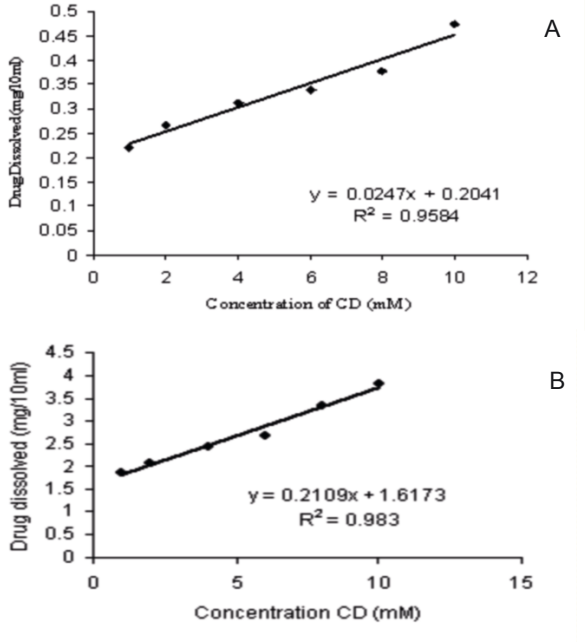


Figure 1. (a) Phase solubility diagram of EDC: HP-β-CD in water at 37°C (n=3) (b) EDC: HP-β-CD in water at 25°C (n=3).

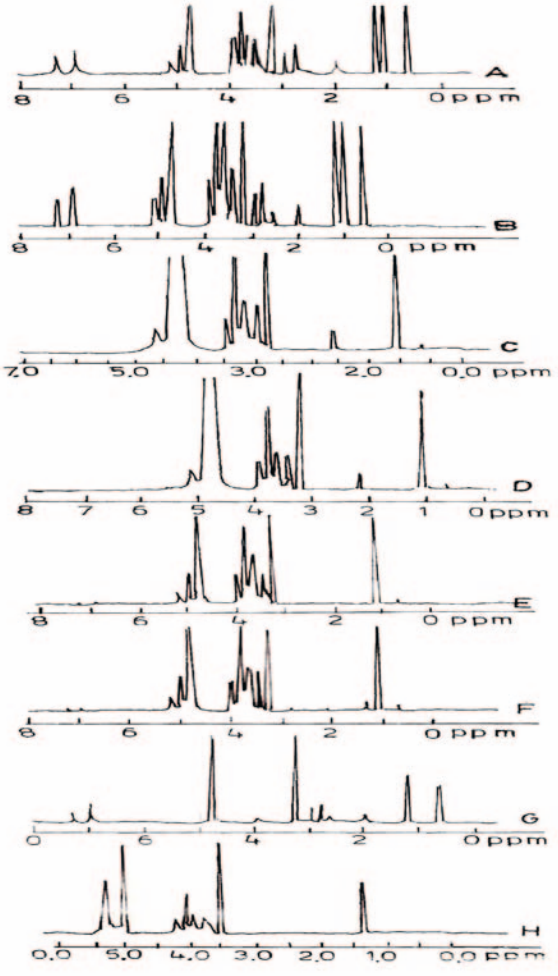


Figure 2. Proton NMR spectrum of A) HPPM1, B) HPPM2, C) KDHP1, D) KDHP2, E) CPHP1, F) CPHP2 G) Pure EDC H) HP-β-CD.

Table 2. Proton Chemical shifts corresponding to HP-β-CD in absence and presence of EDC.

Proton	δ HP-b-CD Free	$\delta_{1:1}$ (KDHP1)	$\Delta\delta^*_{1:1}$ (KDHP1)	$\delta_{1:2}$ (KDHP2)	$\Delta\delta^*_{1:2}$ (KDHP2)	$\delta_{1:1}$ (CPHP1)	$\Delta\delta^*_{1:1}$ (CPHP1)	$\delta_{1:2}$ (CPHP2)	$\Delta\delta^*_{1:2}$ (CPHP2)
H-1	5.0448	5.0933	0.0485	5.0855	0.0407	5.0962	0.0514	5.0855	0.0407
H-2	3.5262	3.5329	0.0067	3.5250	-0.0012	3.5317	0.0055	3.5250	-0.0012
H-3	3.9027	3.9095	0.0068	3.9017	-0.0078	3.9117	0.0009	3.9017	-0.0010
H-4	3.5011	3.5115	0.0104	3.5037	0.0026	3.5061	0.0050	3.5032	0.0021
H-5	3.6475	3.6594	0.0119	3.6516	0.0041	3.6484	0.0009	3.6516	0.0041
H-6	3.7683	3.7773	0.0090	3.7695	0.0012	3.7732	0.0049	3.7695	0.0012
CH_3	1.0670	1.0777	0.0107	1.0699	0.0029	1.0735	0.0065	1.0699	0.0029

$\Delta\delta^* = \delta_{complex} - \delta_{etodolac(free)}$.

Table 3. Proton Chemical shifts corresponding to EDC in absence and presence of HP-β-CD.

Proton	δ EDC Free	$\delta_{1:1}$ (KDHP1)	$\Delta\delta^*_{1:1}$ (KDHP1)	$\delta_{1:2}$ (KDHP2)	$\Delta\delta^*_{1:2}$ (KDHP2)	$\delta_{1:1}$ (CPHP1)	$\Delta\delta^*_{1:1}$ (CPHP1)	$\delta_{1:2}$ (CPHP2)	$\Delta\delta^*_{1:2}$ (CPHP2)
H-3	6.9240	6.9247	0.0007	6.9178	-0.0062	6.9281	0.0041	6.9178	-0.0062
H-4	7.2578	7.2743	0.0165	7.2665	0.0087	7.2678	0.0100	7.2665	0.0087
H-5	6.9557	6.9669	0.0112	6.9495	-0.0061	6.9525	-0.0032	6.9594	0.0037
H-11	2.0078	2.0370	0.0292	2.0339	0.0261	2.0282	0.0204	2.0710	0.0634
H-12	0.6368	0.6456	0.0088	0.6378	0.0010	0.6460	0.0092	0.6469	0.0101
H-13	2.7851	2.7944	0.0093	2.7889	0.0038	2.7929	0.0078	2.7889	0.0038
H-14	1.1971	1.2308	0.0337	1.2230	0.0259	1.2252	0.0281	1.2230	0.0259

$\Delta\delta^* = \delta_{complex} - \delta_{etodolac(free)}$.

However in case of co-evaporated complexes, major chemical shifts were obtained due to presence of protons at position 4, 11 and 14 in aromatic ring of EDC molecule. In case of kneaded complexes (KDHP1 and KDHP2), EDC protons in complex form showed positive $\Delta\delta$ values (downfield) in the range of 0.0007 to 0.0337 for KDHP1 and -0.0062 to 0.0259 for KDHP2 (downfield and upfield) were observed for H-4, H-5, H-11 and H-14 protons. EDC protons in co-evaporated complexes showed $\Delta\delta$ values in range of -0.0032 to 0.0281 for CPHP1 and -0.0062 to 0.0634 for CPHP2. On the basis of these results, proposed molecular 3D representations of the proton interaction between the EDC-HP-β-CD inclusion complexes and their minimized energy structures have been presented in Figs. 3A-3D.

3.3. DSC analysis

DSC thermograms of pure EDC exhibited a sharp endothermic peak at 153°C (Fig. 4E). The thermogram of HP-β-CD (Fig. 4D) showed a very broad endothermic peak in the range of 90.03°C due to elimination of water of crystallization, as reported by many authors [13,14]. The endothermic peak of the drug was retained at 150.9°C in the case of the physical mixture (Fig. 4A). These observations may be attributed to the absence of any significant interaction between the pure components in the physical mixture. The DSC thermogram of KDHP1 displayed an endothermic peak at 57.67°C (Fig. 4B). The endothermic peak corresponding to the melting point of EDC was absent in this case, which may be ascribed to the formation of inclusion complexes. Similar results have previously been observed by various authors [15,16].

The solid complex obtained by the co-evaporation method (CPHP1) showed a broad endotherm at 59.65°C (Fig. 4C). The free drug peak at 153°C was absent, which may be due to interaction between the drug and HP-β–CD. These data suggest that inclusion complexation of drug in HP-β–CD was achieved by the co-evaporation method and that EDC might have formed an inclusion complex with HP-β–CD.

3.4. SEM analysis

Scanning electron microphotographs of the EDC-HP-βCD inclusion complex and of the physical mixture are presented in Figs. 5A-5G. EDC appears as irregular-shaped crystals (Fig. 5A). HP-β-CD shows somewhat spherical shape (Fig. 5B), consistent with the amorphous state of HP-β-CD as observed in the XRD. The physical mixture clearly shows EDC particles with a crystalline structure (Fig. 5C). The EDC: HP-β-CD physical mixture shows crystals of drug along with amorphous HP-β-CD spherical particles. The similarities in morphology

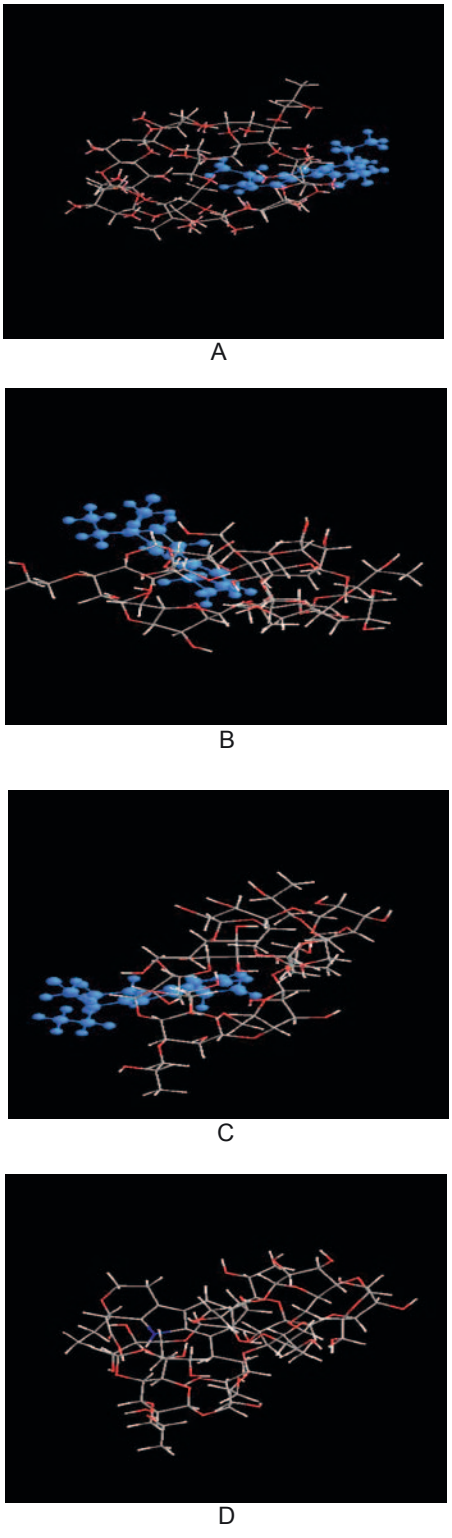


Figure 3. Schematic 3-D molecular representation of inclusion complexes A) Minimized structure of inclusion complex form B), C) EDC-HP-β-CD complexes and proton interaction at different angles and D) 2-hydroxy propyl-β-cyclodextrin moiety.

between these systems and the pure components could be taken as an indication that little EDC: HP-β-CD interaction has taken place in the solid state. A drastic change in the shape and aspect of both components was observed in the inclusion complexes. This change is indicative of the formation of the inclusion complex of EDC with HP-β-CD as the effect of kneading. The features of neither pure substance could be detected in the kneaded complex (Figs. 5D and 5E). This observation suggested the existence of interaction between EDC and HP-β–CD. There were not any considerable differences among the morphologies of kneaded complexes prepared in different molar ratios.

Further, the features of EDC crystals were not easily detectable in the case of co-evaporated complexes (Figs. 5F and 5G). The surface morphologies of the co-evaporated complexes showed bigger agglomerates than were observed in the case of kneaded complexes, and it was difficult to differentiate the drug particles from HP-β-CD particles. This demonstrated that the drug-containing particles had greatly reduced crystallinity, and this was indicated in the XRD patterns as well. This reduced crystallinity is indicative of an interaction of EDC with HP-β–CD.

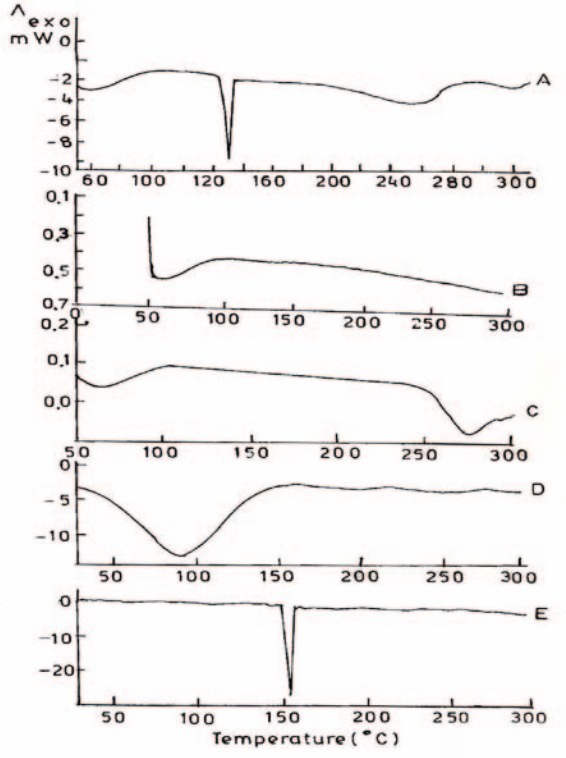


Figure 4. DSC thermogram of A) HPPM1, B) KDHP1, C) CPHP1, D) HP-β-CD and E) Pure EDC.

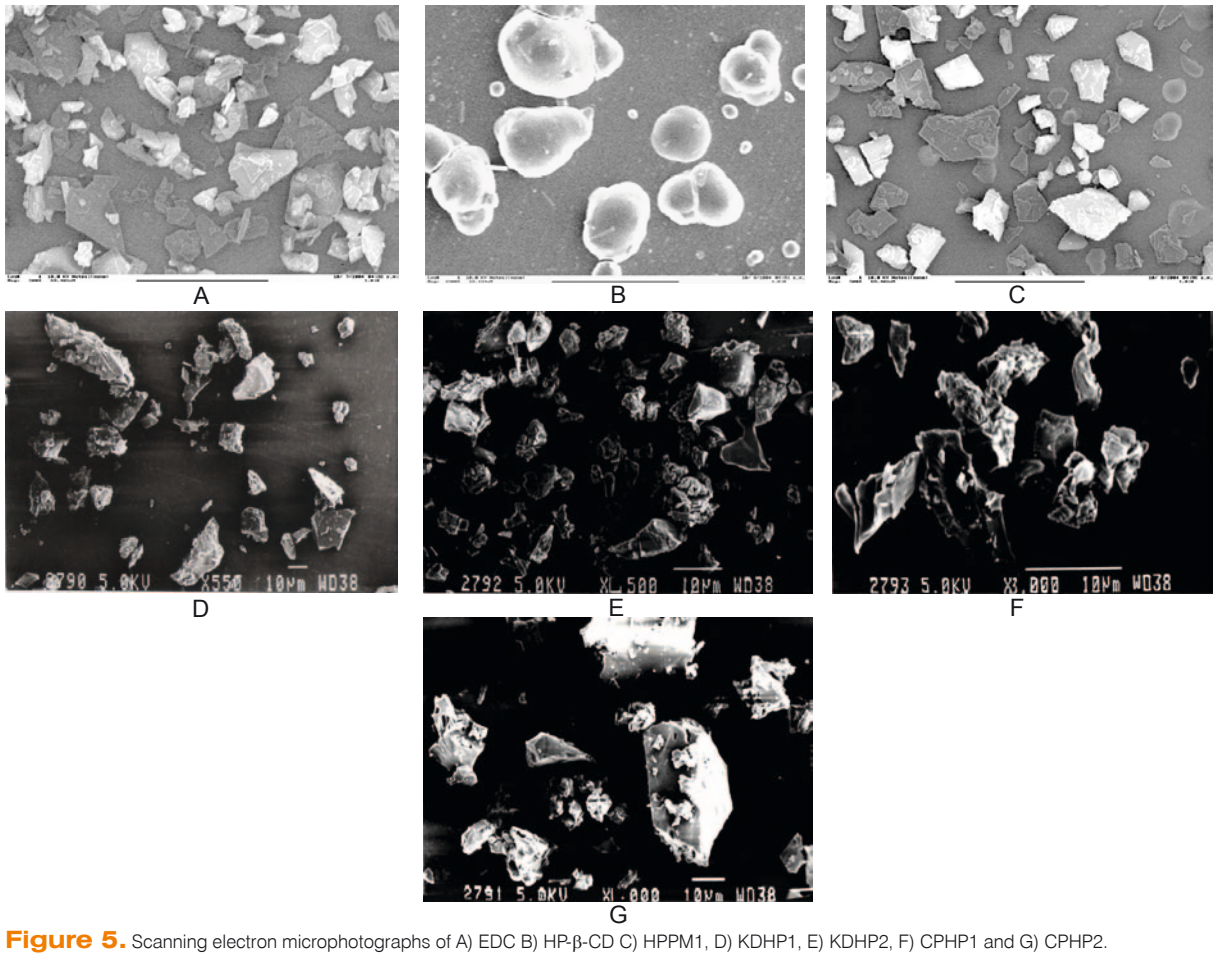


Figure 5. Scanning electron microphotographs of A) EDC B) HP-β-CD C) HPPM1, D) KDHP1, E) KDHP2, F) CPHP1 and G) CPHP2.

3.5. X-Ray Diffractional Studies

The powder X-ray diffractogram of EDC (Fig. 6A) exhibited intense peaks, indicating high crystallinity. HP-β–CD exhibited no sharp peaks due to its highly amorphous nature (Fig. 6B). Most of the principal peaks of EDC were present in the diffraction patterns of physical mixtures of EDC with HP-β–CD. This indicates that there is little or no interaction between the pure components in the case of physical mixtures.

In case of the kneaded complexes prepared using HP-β–CD, the product was quite amorphous in nature (Figs. 6C and 6D). The diffraction pattern was more like that of pure HP-β–CD. There were very few peaks, indicating a loss of crystallinity in the complex, and the pattern was same for both KDHP1 and KDHP2. Only a few peaks were observed and they too were quite different from those observed with pure EDC, which may be indicative of formation of complex. However, in case of the co-evaporated complexes prepared using HP-β–CD, the complexes prepared showed sharp peaks (Figs. 6E and 6F). There were fewer peaks

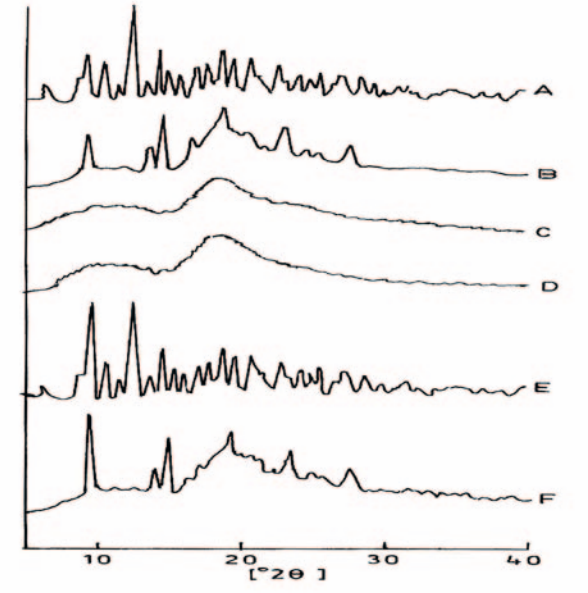


Figure 6. Powder X-ray diffraction pattern of A) EDC, B) HP-β-CD, C) KDHP1, D) KDHP2, E) CPHP1 and F) CPHP2.

than in the diffraction pattern of the pure EDC. There were new peaks which showed the crystalline nature of the complex. The complex was crystalline, unlike the kneaded complexes. It was almost same for both the ratios. Also, the peaks that were observed were quite different from those observed with pure EDC, which indicate the formation of complex.

3.6. FTIR studies

The IR spectrum of EDC showed medium absorption bands at 1740 cm^{-1} characteristic of –COOH and at 3580 cm^{-1} due to single –NH stretching vibration (Fig. 7A). The other characteristic band at 1420 cm^{-1} may be attributed to C-O-C symmetric and asymmetric stretching vibrations. The IR spectrum of HP-β–CD showed a broad absorption band at 3395.9 cm^{-1} due to –OH stretching (Fig. 7B). The IR spectrum of the physical mixture HPPM1 retained the characteristic –NH absorption band. There was no shift in the C=O stretching vibration of EDC at 1740 cm^{-1} (Fig. 7C). These observations led to the conclusion that there was little interaction between EDC and HP-β–CD in the physical mixture. The –NH stretching band of EDC at 3580 cm^{-1} was masked in the complexes. The characteristic –NH stretching band at 3580 cm^{-1} was shifted in all kneaded complexes. It appeared as a single broad peak at 3400 cm^{-1} in case of KDHP1 and KDHP2 (Figs. 7C and 7D). 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 HP-β–CD, which could have resulted from 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 the NH stretching vibration, and masking of C=O bands in kneaded complexes signify the existence of interaction between the drug and HP-β–CD.

The characteristic NH stretching band at 3580 cm^{-1} was shifted in 1:1 and 1:2 co-evaporated complexes (CPHP1 and CPHP2) (Fig. 7E and 7F). It appeared as broad band at 3500 cm^{-1} in case of 1:1 co-evaporated complex (CPHP1) and 3400 cm^{-1} in case of 1:2 co-evaporated complex (CPHP2). The C=O stretching at 1740.0 cm^{-1} was missing in case of the co-evaporated complexes. The broadening of the -NH stretching vibration, and the masking of C=O bands in kneaded complexes signify the existence of interaction between the drug and HP-β–CD.

3.7. In-vitro release studies

In vitro release studies revealed the mean percent release of drug from kneaded complexes KDHP1 and KDHP2 at 30 min (2.08 and 2.20 times as high respectively) relative to pure EDC (Fig. 8). The physical mixtures HPPM1 and HPPM2 also increased the mean percent release of drug by factors of 1.07 and 1.16 respectively. The mean percent release of EDC at 30 min was 93.51%, 98.79% and 44.86% for KDHP1, KDHP2 and free drug respectively, while for the physical mixtures, HPPM1 and HPPM2 it was 48.31% and 52.35% respectively.

Significant increase in the dissolution rates were observed for the co-evaporated complexes (CPHP1 and CPHP2) as compared to their respective physical mixtures and pure EDC (Fig. 8). The mean percent release of drug from co-evaporated complexes CPHP1 and CPHP2 at 30 min. was 2.25 and 1.89 times as high as pure EDC. The physical mixtures HPPM1 and HPPM2 also increased the mean percent release of drug by factors of 1.08 and 1.16 respectively. These data suggest that kneaded and co-evaporated complexes are more effective in increasing the dissolution rate than the physical mixtures. The mean percent release of EDC at 30 min was 100.98%,

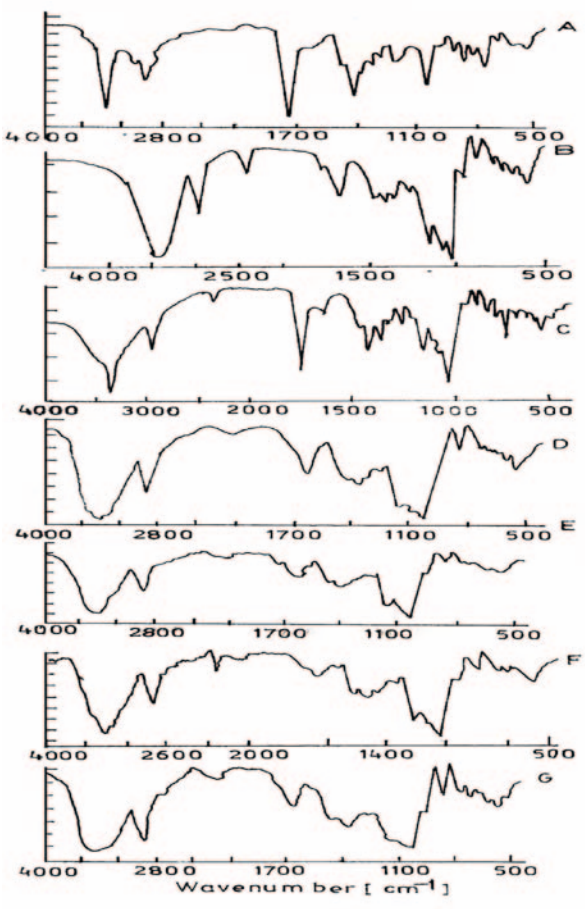


Figure 7. FTIR spectra of A) EDC, B) HP-β-CD, C) HPPM1 D) KDHP1, E) KDHP2, F) CPHP1, G) CPHP2.

84.75 and 44.86% for CPHP1, CPHP2 and free drug respectively while for physical mixture it was 48.31% and 52.35%, respectively.

3.8. Stability Studies

The thermal behavior of the inclusion complexes was analyzed by comparing thermograms of stressed samples with control samples (Fig. 9). The DSC thermogram of KDHP1 and KDHP2 showed one endothermic peak at 94.18°C and 98.70°C associated with the loss of water from HP-β-CD respectively. There was no change in DSC trace of KDHP1 when it was subjected to stress conditions for 3 months, indicating the stability of the complex in stressed condition. However, in case of KDHP2, a small peak corresponding to the melting point of the drug was observed at 160.91°C, which could be due to dissociation of trace amounts of free drug in the complex under these conditions.

3.9. Quantitative assay after an isothermal stress test

In this study the controls as well as the stress samples were analyzed through HPLC for their drug content assay. The drug contents at 1 month, 2 months and 3 months were found to be 103.28%, 104.40%, 107.54% for KDHP1 and 107.95%, 108.17%, 106.84% respectively in case of KDHP2. The data suggested no change in the drug content of the inclusion complexes after three months of the stress conditions and the EDC-inclusion complexes were stable in the stressed conditions without any sign of the degradation of the drug.

4. Conclusions

Complexation of EDC with HP-β–CD led to a stable system with improvement of drug solubility and dissolution rate of hydrophobic candidate EDC. Comparison of the materials prepared with their corresponding physical mixtures revealed the formation of true inclusion complexes, suggesting that an EDC:HP-β–CD complex may be very useful and adaptable in pharmaceutical preparations, with possible enhancement of oral bioavailability.

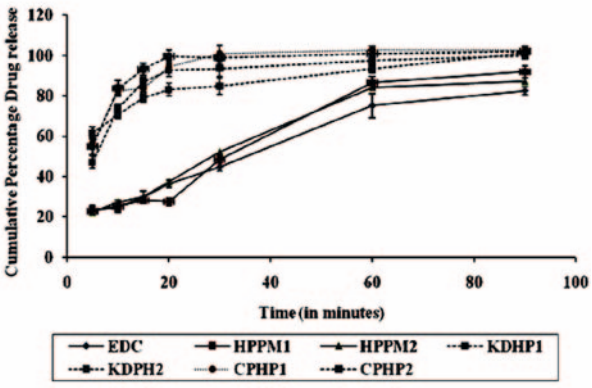


Figure 8. Dissolution profiles of pure drug (EDC), HPPM1, HPPM2, KDHP1, KDHP2, CPHP1 and CPHP2.

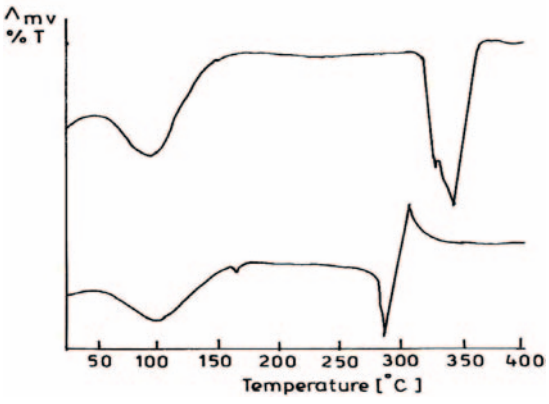


Figure 9. Thermogram for stability analysis of complexes after completion of 3 months A) KDHP1 B) KDHP1.

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