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Comparative Study on Co-Ground Products of Rofecoxib with β-Cyclodextrin and Its Sulfobutyl Ether-7 Derivative in Solution and in the Solid State

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Abstract

The inclusion behavior of sulfobutyl ether-7 derivative of β-cyclodextrin (SBE7βCD), in solution and solid state was compared with that of natural β-cyclodextrin (βCD) toward a poorly water-soluble anti-inflammatory agent, rofecoxib (ROFX), chemically 4[4-(methylsulfonyl) phenyl]-3-phenyl-2 (5H)-furazone. Drug-cyclodextrin solid systems were prepared by cogrinding in a ball mill. A phase solubility method was used to evaluate the stoichiometries and stability constants of ROFX-βCD (1 : 1 and 62 M^{-1}) and ROFX-SBE7βCD (1 : 1 and 132 M^{-1}) complexes. The formation of inclusion complexes with βCD and SBE7βCD in the solid state were confirmed by infrared spectroscopy, differential scanning calorimetry, X-ray diffractometry, scanning electron microscopy and in the liquid state by phase solubility analysis, nuclear magnetic resonance spectroscopy and circular dichroism studies. Dissolution studies using the USP paddle method were carried out in phosphate buffer pH 7.2 at 37 °C for both βCD and SBE7βCD complexes of rofecoxib. Solubility enhancement was much greater for the rofecoxib-SBE7βCD complex compared to drug-βCD complex. The stability constant obtained for the SBE7βCD inclusion complex of rofecoxib was the highest. Finally, dissolution profiles obtained suggest that SBE7βCD is more effective than β-cyclodextrin in improving the pharmaceutical properties of rofecoxib.

Introduction

Rofecoxib (4[4-(methyl sulfonyl) phenyl]-3-phenyl-2 (5H)-furazone), a novel highly selective COX-2 inhibitor is used for a variety of acute and chronic inflammatory diseases. However, low aqueous solubility and poor dissolution of this molecule, delays its rate of absorption and finally the onset of action [1]. In recent years, cyclodextrin complexation has been successfully used to improve solubility, dissolution rate, chemical stability and bioavailability of a number of poorly soluble drugs, including various anti-inflammatory drugs, obtaining in this case further advantages such as dose lowering, reduction of gastric irritation and taste masking [2–3]. The β-cyclodextrins are β-1,4-linked cyclic oligosaccharides composed of seven D-glucopyranose units with a relatively hydrophobic central cavity [4]. These products are able to entrap poorly soluble drug molecules to form reversible noncovalent inclusion complexes. This may improve physical and chemical properties of the incorporated guest molecule allowing, for example, the improvement of solubility, stability [5], *in vivo* drug delivery and bioavailability [6–7]. However, it is known that the application of β-cyclodextrin in the pharmaceutical field is limited by its rather low aqueous solubility, which led to a search for more soluble derivatives of cyclodextrins [4–8]. More recently an anionically charged derivative with an average degree of substitution of 7, sulfobutyl ether 7-β-cyclodextrin (SBE7βCD) has attracted growing interest due to its greater intrinsic solubilities allowing improved complexing abilities [5–9].

Therefore, in the present work it was considered worthy of interest to extend our investigation to this highly water soluble β-cyclodextrin derivative, i.e., sulfobutyl ether-7-β-cyclodextrin, with the aim of evaluating its complexing and solubilizing efficacies toward rofecoxib and comparing them with those of the parent β-cyclodextrin.

Drug-cyclodextrin solid systems were prepared by cogrinding in a ball mill. The formation of inclusion complexes with βCD and SBE7βCD in the solid state was confirmed by infrared spectroscopy, differential scanning calorimetry, scanning electron microscopy and powder X-ray diffractometry. Drug-cyclodextrin interactions in solution were investigated by phase solubility analysis and nuclear magnetic resonance spectroscopy supported by circular dichroism studies while dissolution rates were determined using the USP paddle method.

Materials and methods

Materials

Rofecoxib (ROFX) was a gift sample from Cipla Ltd. (Mumbai, India). β-cyclodextrin, was kindly provided by

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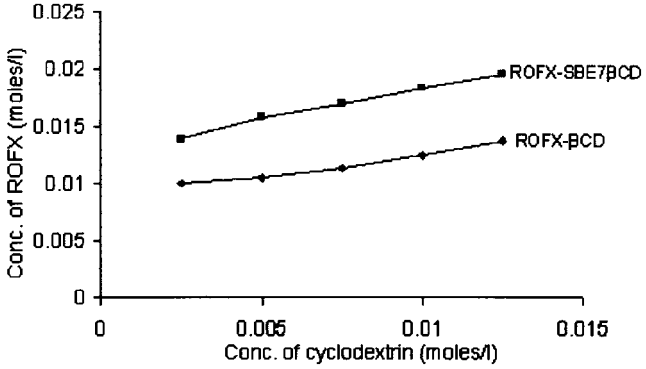


Figure 1. Phase solubility diagram of ROFX-cyclodextrin system.

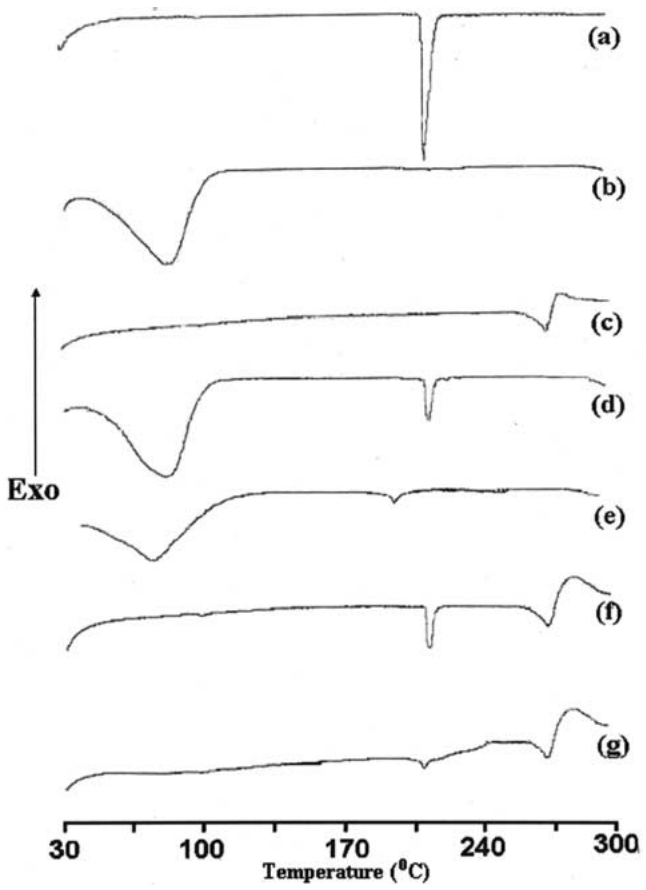


Figure 2. DSC curves of ROFX (a), βCD (b), SBE7βCD (c), ROFX-βCD equimolar physical mixture (d), ROFX-βCD coground product (e), ROFX-SBE7βCD equimolar physical mixture (f), ROFX-SBE7βCD coground product (g).

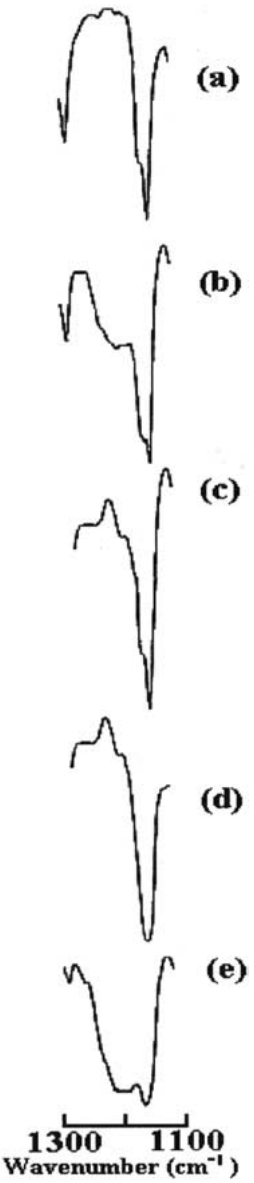


Figure 3. FTIR spectra of ROFX (a), ROFX-SBE7βCD equimolar physical mixture (b), ROFX-βCD equimolar physical mixture (c), ROFX-βCD coground product (d), ROFX-SBE7βCD coground product (e).

S.A. Chemicals (Mumbai, India) and sulfobutyl ether-7β-cyclodextrin was generously donated by Cydex Inc. (Overland Park, KS). All reagents and solvents used were of analytical grade.

Phase solubility studies

Solubility measurements were performed according to Higuchi and Connors [10]. Excess amounts of drug were added to 10 ml of CD solutions in phosphate buffer pH 7.2 (0.003–0.015 M concentration range) in 25 ml stoppered conical flasks and shaken at 25 ± 0.5 °C. At equilibrium after 2 days, aliquots were withdrawn, filtered (0.45 μm pore size) and spectrophotometrically assayed for drug content at 228 nm (Shimadzu-UV 160A Spectrophotometer). Each experiment was carried out in triplicate. (coefficient of variation, C.V. < 3%). The apparent 1 : 1 stability constants of the ROFX-CD complexes were calculated from the slope and intercept of the straight lines of the phase-solubility diagrams according to the following equation:

$$\text{Kc} = \text{Slope/So (1-Slope)},$$

where Kc is an apparent stability constant/complexation constant assuming a 1 : 1 stoichiometry, So is the intrinsic solubility of the compound in the absence of complexing agent [11].

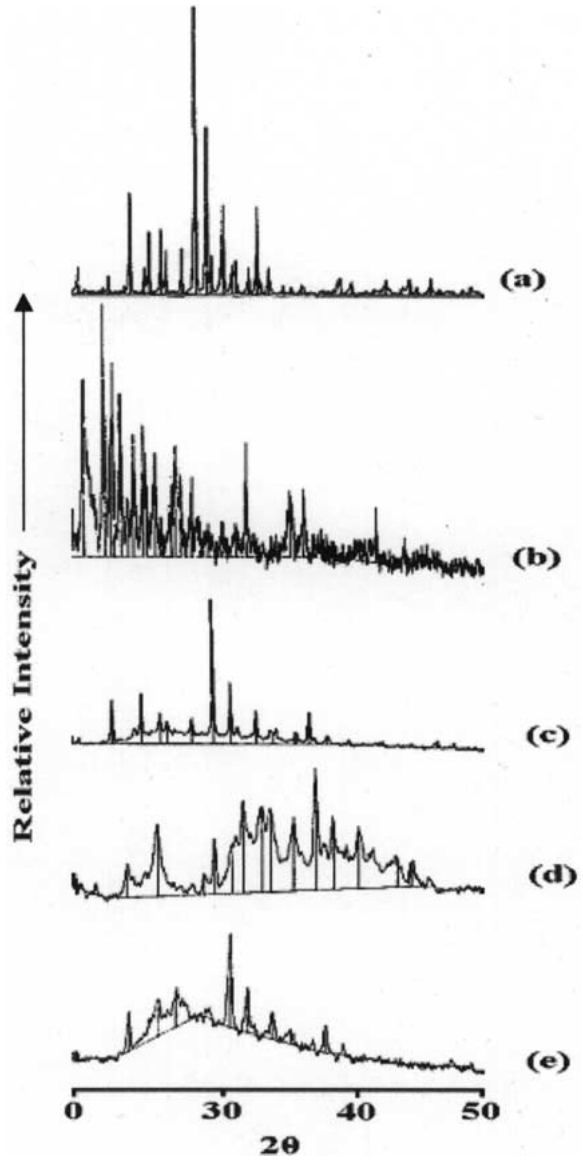


Figure 4. XRD spectra of ROFX (a), ROFX-βCD equimolar physical mixture (b), ROFX-SBE7βCD equimolar physical mixture (c), ROFX-βCD coground product (d), ROFX-SBE7βCD coground product (e).

Preparation of solid inclusion complexes

Cogrinding method was used for the preparation of drug-cyclodextrin solid systems. ROFX is mixed with β-cyclodextrin and sulfobutyl ether-7-β-cyclodextrin in the 1 : 1 molar ratio. Coground products were prepared by 30 mm ball-milling physical mixtures at 50 rpm for 12 h. Each solid product was sieved through 100-mesh sieve and the same fraction was used for the following tests.

Differential scanning calorimetry (DSC)

A Shimadzu-Thermal Analyzer DT 40 was used for recording DSC thermograms of the rofecoxib raw material, of inclusion complexes prepared by cogrinding and of the physical mixtures. Samples (2–8 mg) were accurately weighed using a Sartorius 4503 electronic microbalance and heated in closed aluminium crimped cells at a rate of 10 °C/min in the 30–300 °C temperature range under a nitrogen flow of 40 ml/min. Reproducibility was checked by running the sample in triplicate.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform IR spectra were recorded on a Jasco FTIR-5300 spectrophotometer. The spectra were recorded for rofecoxib, βCD, SBE7βCD, physical mixtures and their coground complexes. Samples were prepared in KBr disks (2 mg sample in 200 mg KBr) by means of hydrostatic press at a force of 5.2 τ cm^{-2} for 3 min. The scanning range was 450–4000 cm^{-1} and the resolution was 4 cm^{-1}.

X-ray powder diffractometry

X-ray powder diffraction patterns of rofecoxib raw material, the inclusion complexes and the physical mixtures were recorded on a Philips PW 3040/60 powder X-ray diffractometer using Ni-filtered, CuKα radiation, a voltage of 40 kV and a 25 mA current. The scanning rate employed was 1° min^{-1} over the 0–50° diffraction angle (2θ) range.

Scanning electron microscopy (SEM) analysis

SEM analysis was carried out using a Jeol JSM-840 scanning microscope. Prior to examination, samples were gold sputter-coated to render them electrically conductive.

Nuclear magnetic resonance (NMR) spectroscopy

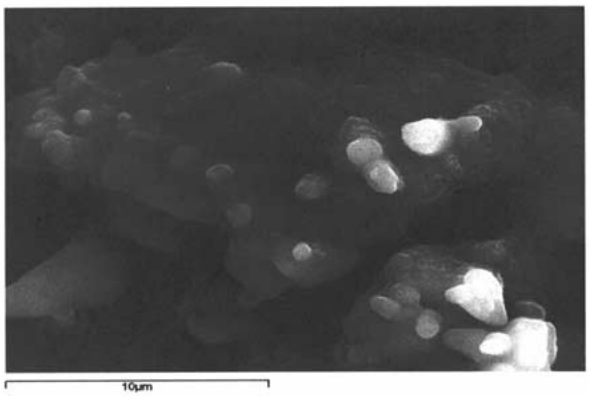
^{1}H-NMR spectra of rofecoxib and its complexes with βCD and SBE7βCD were recorded using a Bruker AVANCE 500 DRX (500 MHz) instrument. The samples for NMR measurement were prepared in 0.6 ml of 0.1 M NaOD in D_2O.

Circular dichroism spectroscopy

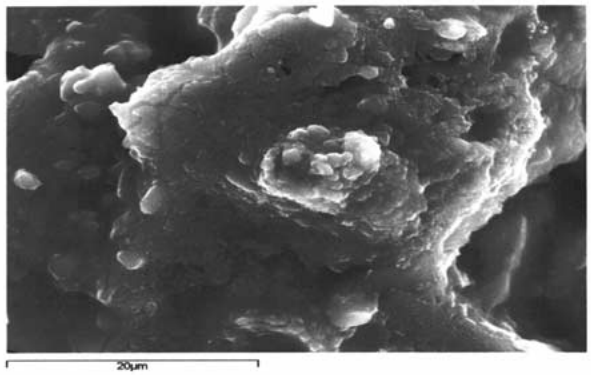
Circular dichroism spectra were obtained by a Jasco J-600 Spectropolarimeter. Absorbances of the samples were kept below 2 in the whole wavelength range explored (230–350 nm). All the spectra were corrected for the signal exhibited by the βCD and SBE7βCD solution in the absence of the guest. The signal-to-noise ratio was improved by superposition of five different scans.

Dissolution rate studies

Dissolution rate experiments were performed in phosphate buffer pH 7.2 at 37 $\pm$ 0.5 °C, using USP XXI/XXII apparatus (Electrolab, India) with paddle stirrer rotating at 50 rpm. Solid products, each containing 50 mg of drug, were subjected to dissolution. At fixed time intervals, samples were withdrawn with a filter syringe (pore size 0.45 μm) and assayed spectrophotometrically for drug content at 228 nm. Each test was carried out in triplicate (coefficient of variation (C.V.) $<$ 3%). Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as percentage of



(a)



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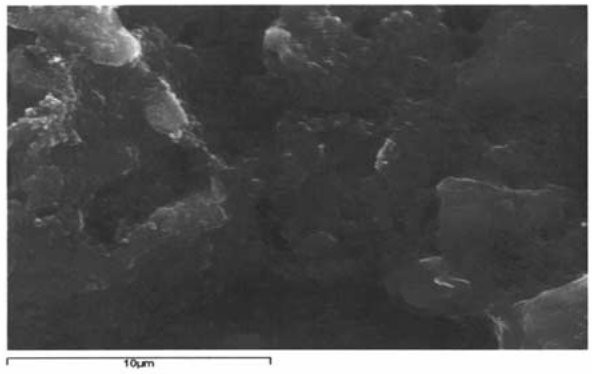
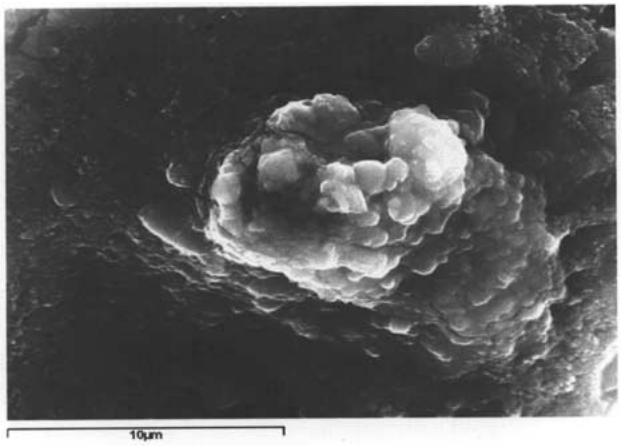
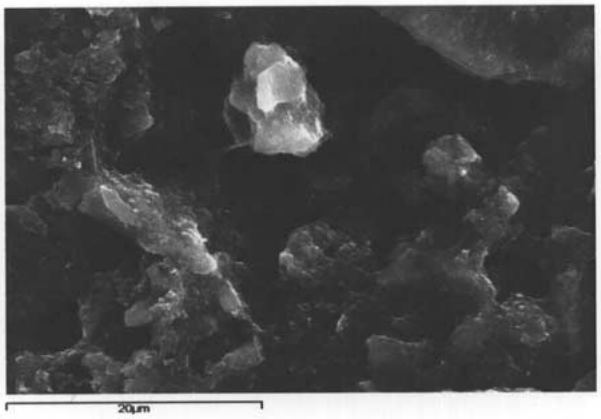


(c)

Figure 5a–c. SEM photographs of ROFX (a), ROFX-SBE7βCD complex (b), ROFX-βCD complex (c), ROFX-SBE7βCD physical mixture (d), ROFX-βCD physical mixture (e), SBE7βCD (f), βCD (g).



(d)



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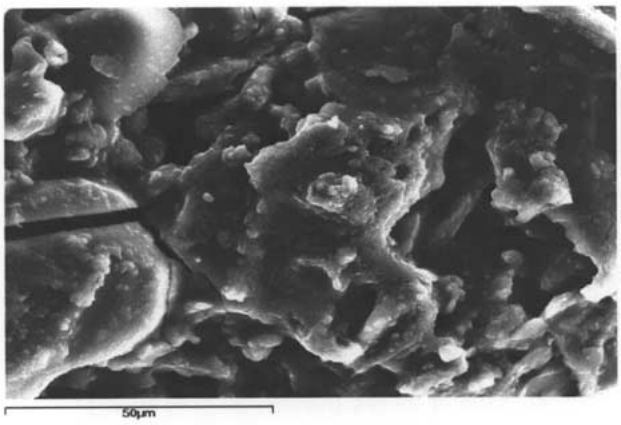

(f)

Figure 5d–f.

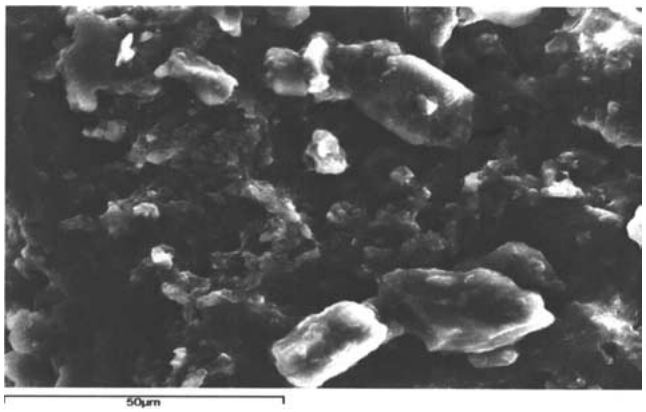

Figure 5g.

the area of the rectangle described by 100% dissolution in the same time [12].

Results and discussion

Solubility studies

Phase solubility diagrams of ROFX-βCD and ROFX-SBE7βCD are shown in Figure 1. They showed a linear relationship between the amount of ROFX solubilized and the concentration of cyclodextrin in solution. According to Higuchi and Connors [10], this may be attributed to the formation of soluble 1 : 1 ROFX-cyclodextrin inclusion complexes. Solubility enhancements were much greater with SBE7βCD as compared to that of parent βCD. Stability constant obtained for SBE7βCD (132 M^{-1}) was higher than that for the parent βCD (62 M^{-1}).

Solid-state studies

Differential scanning calorimetry

The thermal curves of pure components and of the different drug-cyclodextrin systems are shown in Figure 2. The DSC curve of ROFX was typical of a crystalline anhydrous substance, with a sharp fusion endotherm (T_{onset} = 207.9 °C, T_{peak} = 211.3 °C). Liberation of crystal water from βCD (14.5% as mass fraction) was observed as a broad endothermal peak at around 100 °C. SBE7βCD is an amorphous material and does not exhibit a melting point as would be observed for crystalline materials. The DSC thermogram shows a broad endotherm from approximately 40 to 150 °C, consistent with dehydration of the sample. Decomposition events begin at approximately 260 °C. The characteristic, well recognizable thermal profile of the drug appeared at the temperature corresponding to its melting point in the physical mixtures of drug with both βCD and SBE7βCD. However, the thermal profile of the drug appeared at lower temperatures, strongly reduced in intensity and somewhat broadened in the coground products ROFX-βCD and ROFX-SBE7βCD. This phenomenon, assumed as a proof of interactions between the components of the respective binary systems can be considered as indicative of drug amorphization and/or inclusion complex formation [13].

Infrared spectroscopy

Infrared spectra of ROFX, as well as those of its solid systems with cyclodextrins, are presented in Figure 3. The peak at 1149 cm^{-1} of the O=S=O stretching of the sulfonyl group was the important characteristics of ROFX. The characteristic sulfonyl stretching band of pure drug appeared unchanged in the physical mixtures of both cyclodextrins. But it appeared broader and shifted to a higher frequency; 1163 cm^{-1} in ROFX-SBE7βCD and 1159 cm^{-1} in ROFX-βCD coground products. This effect could be attributed to the breakdown of the intermolecular hydrogen bonds of the crystals associated to the inclusion of the drug into the hydrophobic cavity of the carrier [14].

X-ray diffraction

Figure 4 shows the X-ray diffraction patterns of ROFX and corresponding complexes with CDs. In the X-ray diffractogram of ROFX powder, sharp peaks at a diffraction angle (2θ) of 15.97°, 22.22°, 23.29°, 24.84°, 28.11° are present and that suggests the drug is present as a crystalline material. Peaks due to drug crystallinity were still detectable in the respective physical mixtures with βCD and SBE7βCD. A total drug amorphization was instead induced by cogrinding: the X-ray diffraction patterns of ROFX-CD systems were characterized only by large diffraction peaks in which it is no longer possible to distinguish the characteristic peaks of rofecoxib. These results indicate that ROFX is no longer present as a crystalline material and its CD solid complexes exist in the amorphous state.

Scanning electron microscopy

Scanning electron photographs presented in Figure 5 reveals the particle morphology of pure compounds ROFX, SBE7βCD and βCD. ROFX appeared as irregular shaped crystals tending to form aggregates. SBE7βCD consisted of irregular three-dimensional particles with parallelogram shape, whereas βCD appeared as irregularly shaped crystals. The physical mixtures showed particles of SBE7βCD and βCD embedded with ROFX particles and a comparable morphology with pure compounds taken separately. In contrast, a drastic change in the morphology and shape of particles was observed in 1 : 1 ground products of both SBE7βCD and βCD, revealing an apparent interaction in the solid state.

CD spectroscopy

ROFX shows a characteristic UV absorption spectrum due to the presence of chromophores but alone it gave no CD band at experimental conditions studied, because it has no asymmetric carbon atom in the molecule. When βCD or SBE7βCD was added to the ROFX solution, a Cotton effect was observed in the wavelength range of 230–350 nm. As seen in Figure 6, in the presence of SBE7βCD, rofecoxib showed a positive peak at 315 nm and relatively

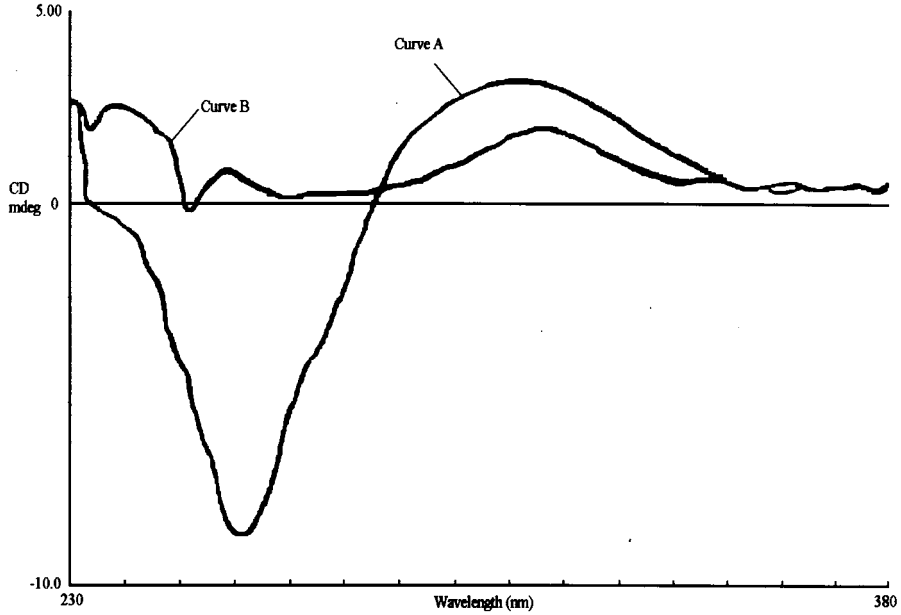

Figure 6. CD spectra of ROFX-Cyclodextrin system. Curve A: ROFX-SBE7βCD system. Curve B: ROFX-βCD system. Concentration: ROFX (8×10^{-5} M). Solvent: Distilled water.

Table 1. Chemical shifts (ppm) for the protons of ROFX in the free state and in the pure inclusion complex with βCD and SBE7βCD

H rofecoxib	δ_{free}	$\delta_{complex}$ βCD	SBE7βCD	$\delta_{complex} - \delta_{free}$ βCD	SBE7βCD
a	7.709	7.646	7.652	−0.063	−0.057
b	7.411	7.384	7.374	−0.027	−0.037
c	7.069	7.010	7.029	−0.059	−0.019
d	7.148	7.103	7.101	−0.045	−0.047

strong negative peak at 260 nm as a result of perturbation of the electronic transition of the drug caused by the asymmetric cavity of cyclodextrin following complexation. The Induced Circular Dichroism (ICD) signals for ROFX-βCD complex is very weak as βCD perturbed strongly longer wavelength region of ROFX to give positive peak at 315 nm and relatively very small negative peak at 260 nm. The more intense spectral modifications observed in the presence of SBE7βCD can be considered the effect of a stronger interaction of SBE7βCD with ROFX in confirmation of the results of phase solubility studies and high polarizability of O—S bond or to the different conformation of SBE7βCD [15].

It is well known that cyclodextrins have neither CD nor absorption band at wavelengths longer than 220 nm and the inclusion of optically inactive compounds within the cyclodextrin cavity generates extrinsic Cotton effect in the wavelength region of drug chromophores. Thus, the CD spectroscopic data indicate that ROFX is embedded in the asymmetric locus of the SBE7βCD and βCD cavities and the configuration of ROFX within the cavity of βCD is somewhat different from that of SBE7βCD.

NMR spectroscopy

Evidence of inclusion complex formation for the ROFX-βCD and ROFX-SBE7βCD system was obtained by NMR spectroscopy. ROFX is composed of two phenyl groups attached to a furazone moiety. All the proton signals except the aromatic protons in the ^{1}H NMR spectrum of pure ROFX are essentially located in a narrow range (4.5–4.8), which makes proton assignments difficult. However, it was observed that in the ^{1}H NMR spectrum of ROFX-CD solid complex (Figure 7), the signals of the aromatic protons of two ROFX phenyl groups showed a considerable spectral shift and as such there are no new peaks that could be assigned to the complexes as shown in Table 1. This might suggest that the drug molecule interacts with the cyclodextrin cavity providing an inclusion complex.

Dissolution rate studies

The dissolution curves of ROFX alone and of the binary systems with βCD and SBE7βCD are presented in Figure 8. The results in terms of dissolution efficiency and percent of active ingredient dissolved are collected in Table 2. It is evident that the binary systems with CDs exhibited faster dissolution rates than ROFX alone. As for the influence

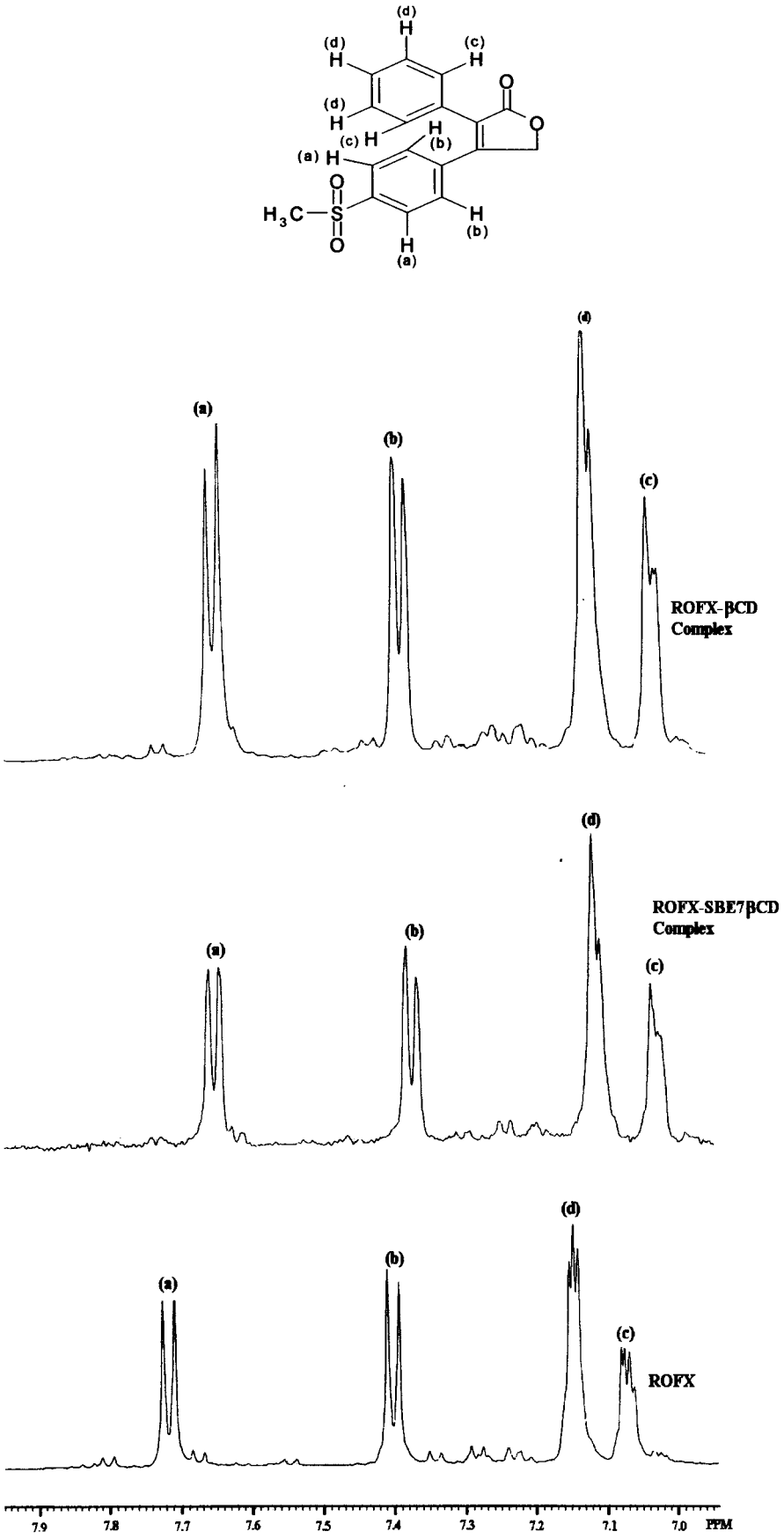

Figure 7. ^{1}H NMR spectra of free ROFX and ROFX-CD complexes.

Table 2. Dissolution parameters of rofecoxib (ROFX) alone and its equimolar physical mixtures (P.M.), coground (GR) products with βCD and SBE7βCD

Sample		DP_{30}	DE_{60}
ROFX	–	26.50	25.08
R + βCD	P.M.	35.36	30.15
	GR	48.74	46.64
R + SBE7βCD	P.M.	38.56	34.02
	GR	73.40	71.58

DP_{30}: Percent drug dissolved at 30 min.
DE_{60}: Dissolution efficiency at $t = 60$ min.

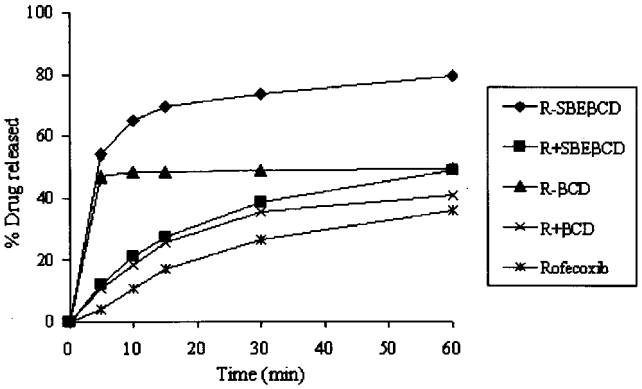

Figure 8. Dissolution curves of ROFX alone, from physical mixtures (R+SBE7βCD, R+βCD) and coground products (R−SBE7βCD, R−βCD).

of the individual cyclodextrin, the greatest improvement of the drug dissolution properties was obtained with coground products of SBE7βCD. Concerning the significant enhancement of the dissolution rate that occurred with coground products, this may be attributed to an increase of solubility upon complexation, to the amorphous state generally occurring during cogrinding (i.e., a decrease in crystallinity), and to reduction in the particle size resulting in an increase of the surface area of the drug, according to the Noyes–Whitney equation [16]

$$dC/dt = D/h \times S(Cs - Ct),$$

where dC/dt is the dissolution rate, D is the diffusion coefficient of the drug, h is the thickness of the diffusion layer, S is the surface area of the dissolving solid, Cs and Ct are the aqueous solubility and the concentration of the drug in the aqueous medium at time t, respectively.

The improvement of dissolution rate obtained with physical mixtures can be attributed to both improved drug wettability and formation of readily soluble complexes in dissolution medium. Table 2 summarizes the various dissolution parameters of rofecoxib and its binary systems with cyclodextrins. The better performance of coground products with SBE7βCD were characterized by dissolution efficiencies 1.5 times higher than those of the coground products with βCD and 3.0 times higher than that of ROFX alone. This can be ascribed to the higher water solubility and amorphous nature of the sulfobutyl derivative of βCD; hence, the higher solubility of ROFX is due to its deeper interactions with SBE7βCD, as confirmed by DSC, X-ray diffraction, and FTIR analysis.

Conclusion

It was shown that the properties of ROFX-cyclodextrin systems are influenced by the type of cyclodextrin. The use of several different physicochemical characterization methods enabled us to fully characterize and evaluate the products and compare their properties in depth. Sulfobutyl ether 7-β-cyclodextrin showed greater solubilizing and amorphizing power toward ROFX than the parent βCD due to its higher water solubility and amorphous nature. Coground systems with SBE7βCD gave satisfactory results, showing dissolution efficiencies 1.5 times higher than the parent βCD and about 3.0 times higher than the pure drug. Thus, sulfobutyl ether 7-β-cyclodextrin can be considered as the most effective carrier for enhancing solubility and dissolution. Grinding technique being industrially applicable seemed to be of great interest and utility if one needs to obtain a simple increase in ROFX solubility and dissolution.

Acknowledgements

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