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Complexation of the Non-steroidal Anti-inflammatory Drug Loxoprofen with Modified and Unmodified β-Cyclodextrins

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Abstract

The inclusion complex of the anti-inflammatory drug, loxoprofen, with β-cyclodextrin-(CD), sulfated β-CD, and glycerol ether β-CD was studied by UV-VIS absorption and ^{1}H-NMR spectroscopy in solution. The inclusion complex of loxoprofen with β-CDs was prepared by freeze-drying, and then characterized in the solid state by thermal analysis, X-ray diffraction, FT-IR and FT-Raman spectroscopy, and scanning electron microscopy (SEM). Furthermore, a physical mixture of loxoprofen/β-CD (1/1, mol-%) in the solid state was also characterized. The solubility of the loxoprofen increased on addition of β-CDs. The solubility enhancement of the loxoprofen with β-CDs is in the following order: glycerol ether β-CD > sulfated β-CD > β-CD.

Introduction

Loxoprofen, α-methyl-4-[(2-oxocyclophenyl-methyl]benzeneacetic acid, is one type of non-steroidal anti-inflammatory drug and a weak acid poorly soluble in water. As a typical anti-inflammatory drug, loxoprofen has been used since 1981 [1]. The preparation, metabolism, and application of loxoprofen has been reported in detail [2–5].

Conversion of poorly water soluble crystalline drugs into an amorphous state is the approach used to improve the biopharmaceutical properties of the oral dosage of a solid. Drug amorphization can be achieved in the molecular dispersion state by grinding the drug with pharmaceutical additives (cellulose, chitin or chitosan, cyclodextrins, polyvinylpyrolidine, etc.) with different amounts of additive and grinding time [6, 7].

As some natural cyclodextrins, especially β-CD, show low aqueous solubility and a toxic effect when used in parenteral application, many efforts have been directed to the development of new cyclodextrin derivatives with better properties. Recently, we reported that glyceryl-linked cyclodextrin can host *p*-nitrophenol, and evidence of inclusion formation of the *p*-nitrophenol with cyclodextrins was obtained using Raman spectroscopy [8].

Generally, complexation with cyclodextrin has been widely used to improve both the dissolution rate and absorption of poorly soluble drugs [9]. The complexes of carboxylic acid group-containing anti-inflammatory agents in cyclodextrin were prepared in order to increase their solubility [10–13]. However, the inclusion of the anti-inflammatory drug, loxoprofen, in modified and unmodified β-cyclodextrin has not been reported yet, to our knowledge.

In this study, the inclusion complex of loxoprofen with β-CD, sulfated β-CD, and glycerol ether β-CD was prepared by mixing and shaking in solution, and then was freeze-dried to obtain the solid products. The physical mixtures of loxoprofen and β-CD, sulfated β-CD, and glycerol ether β-CD were also prepared. The inclusion complex of loxoprofen with β-CDs in the solution state was characterized by UV-VIS absorption and ^{1}H NMR spectroscopy. Furthermore, the inclusion complex and the physical mixture were also characterized by X-ray diffraction, IR spectroscopy, thermal analysis, and scanning electron microscopy (SEM) in the solid state.

Experimental

Materials

The β-cyclodextrin (CD) and sulfated β-CD were obtained from Aldrich Co. The loxoprofen was received from Kolon Co. (Korea). The glycerol ether β-CD was prepared by the following method: 4.0 mmol β-CD was dissolved in 0.05 mmol/mL NaOH solution. 8.0 mmol epichlorohydrin was added slowly dropwise at 60 °C under stirring. After 60 minutes, the reaction was stopped by the addition of acetone/methanol (1/1, vol-%), and then washed with MeOH until the MeOH solution reached pH = 7.0 [8, 14]. The glycerol ether β-CD obtained was dried in vacuo at 50 °C for 3 hrs. The polymer obtained had the general formula [β-CD-$(CH_2$-CHOH-O$)_n$X$]_p$, where X is H or CD; p is >1 and

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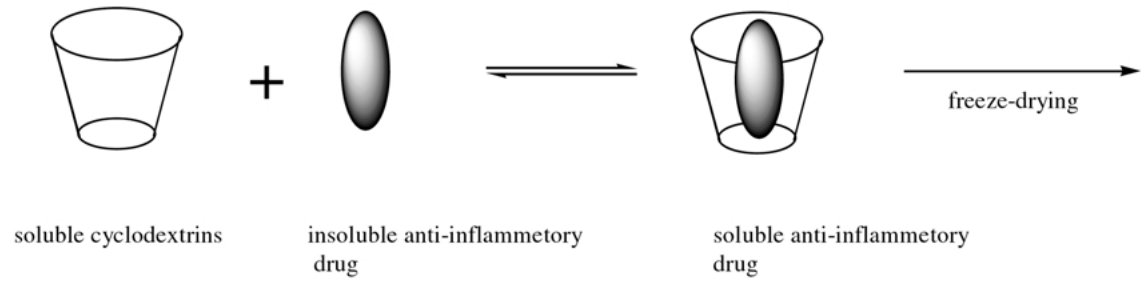

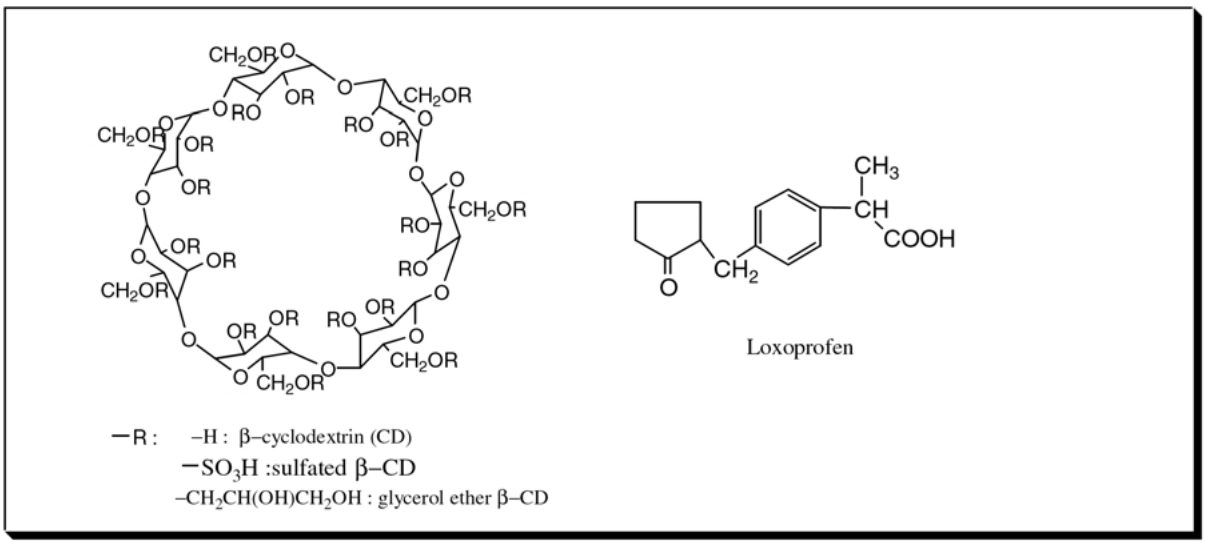

Scheme 1. Inclusion formation of loxoprofen within cavity of the β-CDs.

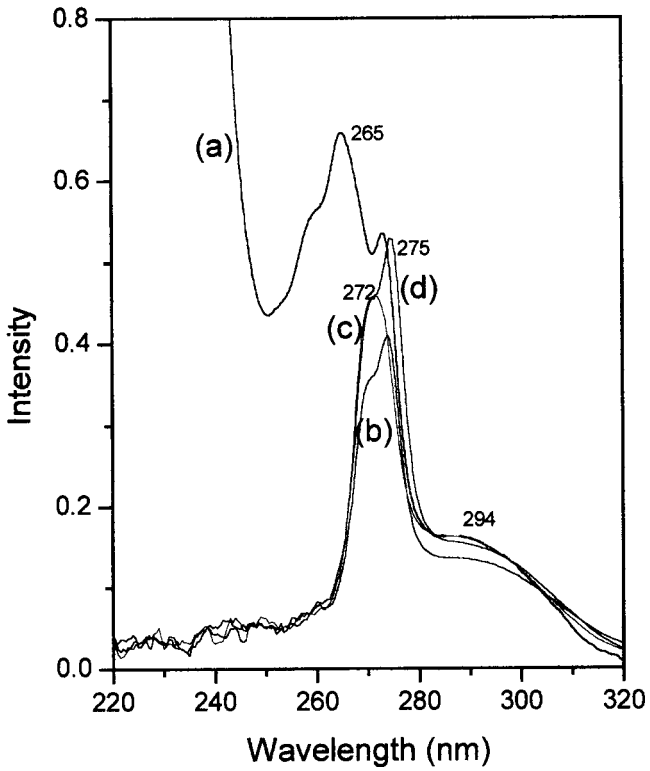

Figure 1. UV-visible spectra of loxoprofen (0.40 mg/mL) in the presence of (a) NaOH (40 mg), (b) β-CD (400 mg), (c) sulfated β-CD (450 mg), and (d) glycerol ether β-CD (170 mg).

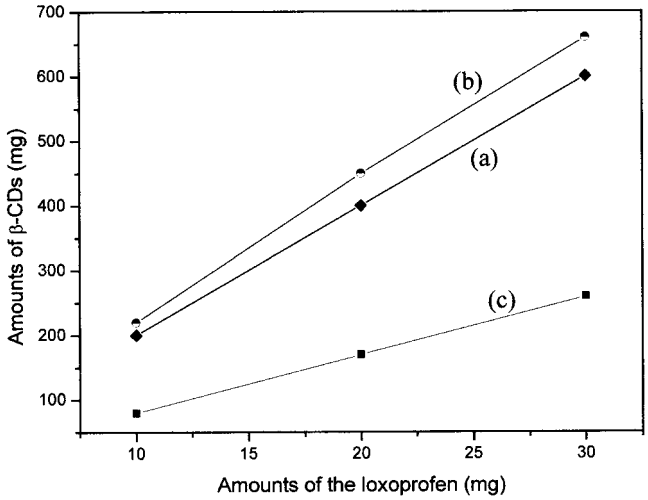

Figure 2. Solubility of loxoprofen in water (50 mL) in the presence of β-CD (a), sulfated β-CD (b), and glycerol ether β-CD (c).

the average *n* value is 12–15. Other chemical reagents were used without purification.

Preparation of the inclusion complex and physical mixture of loxoprofen with β-CDs

Scheme I shows the loxoprofen as guest compound and β-CDs as host compound. The inclusion complex was obtained

by mixing the appropriate amounts of loxoprofen with β-CD, sulfated β-CD, and glycerol ether β-CD in distilled water, and shaking for 24 h at room temperature. The molar ratio of loxoprofen/β-CDs are described in Figure 2. The inclusion complex was frozen and dried by immersion in a shell freezer-drier for over 24 h. The physical mixture of the loxoprofen/β-CDs (1/1, mol-%) was prepared by simple blending.

The solubility of the loxoprofen was determined by adding β-CD, sulfated β-CD, and glycerol ether β-CD in 50 mL H_2O at room temperature, respectively.

Characterization of the inclusion complex and physical mixture

Absorption spectra of the loxoprofen complexes with β-, sulfated β-, and glycerol ether β-CD in H_2O were recorded with a JASCO V-560 UV/VIS spectrophotometer. Furthermore, the pure loxoprofen Na salt was also studied.

One dimensional ^{1}H NMR spectra (400 MHz for ^{1}H) of the loxoprofen complexes were recorded on a Varian spectrometer in D_2O. The loxoprofen Na salt in D_2O was also recorded.

The NIR Fourier transform (FT) Raman spectra were recorded with a Bruker FT-106 Raman module, equipped with a Ge detector cooled by liquid nitrogen and connected to a Bruker FT-IR 66 interferometer. In order to excite the Raman spectra, a continuous wave diode-pumped Nd : YAG laser with radiation of wavelength 1064 nm (9398.4 cm^{-1}) was used. In all cases, the laser power was 300 mW and the spectral resolution 2 cm^{-1}.

FT-IR spectra of the inclusion complexes in the solid state were obtained using Nujol mulls with a Perkin-Elmer Model 983 infrared spectrophotometer.

X-Ray diffraction data were collected on an Enraf-Nonius powder diffractometer equipped with a horizontal mounted INEL CPS120 curved positional-sensitive detector. Cu–$K_{\alpha 1}$ radiation was selected by a bent quartz-crystal monochromator and each pattern was recorded during 24 h.

For scanning electron microscopy (SEM), the sample was coated with gold-palladium alloy prior to the measurement. The sputtered sample was then scanned by the electron beam in the scanning electron microscope (JSM-840A, JEOL Co., Japan).

The differential scanning calorimeter (DSC) traces were measured on a Perkin Elmer DSC-7 Series Thermal Analysis System with a heating rate of 10 °C/min in nitrogen in the temperature range of 50–225 °C. Thermogravimetric analysis (TGA) was carried out on the TA instruments of the TGA 2950 model (Dupont Co.) with a heating rate of 10 °C min^{-1} in nitrogen in the temperature range of 50–700 °C.

Results and discussion

Inclusion complexes of loxoprofen with β-CD, sulfated β-CD, and glycerol ether β-CD in solution

On inclusion of a molecule within a cyclodextrin cavity, the UV-visible spectrum usually changes since the solvation shell of the molecule is partly or totally replaced by the cyclodextrin molecule, leading to an altered solute/environment interaction. Valero *et al.* [15] reported on the absorption and emission spectra of *Nabumetone* with α-, β-, and hydroxypropyl β-CD. They pointed out that the inclusion modes of the *Nabumetone* with the three cyclodextrins were similar. However, a UV-visible study of loxoprofen included in β-CDs has not been reported yet.

Figure 1 shows the UV-visible spectra of the loxoprofen sodium salt (a), the β-CD complex (b), the sulfated β-CD complex (c), and the glycerol ether β-CD complex (d) in aqueous solution. In order to dissolve loxoprofen in H_2O, the loxoprofen was treated with NaOH in H_2O. The λ_{max} of loxoprofen in NaOH solution was at 265 nm due to the $\pi \rightarrow \pi^*$ transition in the aromatic group. On the other hand, the λ_{max} of the loxoprofen included in β-CD, sulfated β-CD, and glycerol ether β-CD was at 275, 272, and 275 nm respectively due to the $\pi \rightarrow \pi^*$ transition in the aromatic group of loxoprofen. The λ_{max} of the inclusion complex was shifted to a longer wavelength (a red shift) due to inclusion of the aromatic group of loxoprofen within the cavity of the β-CDs.

Figure 2 summarizes the solubility of loxoprofen included in β-CD (a), sulfated β-CD (b), and glycerol ether β-CD (c). In order to dissolve 30 mg of loxoprofen, 600 mg of β-CD, 660 mg of sulfated β-CD, and 260 mg of glycerol ether β-CD were used. The molar ratios of loxoprofen/β-CD and loxoprofen/sulfated β-CD are 1/4.2 and 1/2.0, respectively. On the other hand, the molar ratio of loxoprofen/glycerol ether β-CD is 1/0.6. The glycerol ether β-CD used in this study was $[\beta\text{-CD-}(CH_2\text{-}CHOH\text{-}O)_nX]_p$, which has $n = 13$, X = H, and $p = 2$ [8]. The concentration of loxoprofen was 2.5×10^{-3} M.

^{1}H-NMR spectroscopy was used to study the inclusion phenomena of loxoprofen within β-CDs in solution. Analysis of the ^{1}H NMR spectra is especially good for characterizing the inclusion complex because it is soluble in D_2O. However, pure loxoprofen is not soluble in D_2O. In order to dissolve in D_2O, the loxoprofen was treated with NaOH.

Figure 3 shows the ^{1}H-NMR spectra of loxoprofen sodium salt (a), the β-CD complex (b), the sulfated β-CD complex (c), and the glycerol ether β-CD complex (d) in D_2O. The aromatic proton peaks in the ^{1}H-NMR spectra of loxoprofen are observed in the 6.62–7.20 ppm region. The aromatic proton peaks were shifted to a larger chemical shift due to inclusion formation. Gloria *et al.* [15] reported the chiral recognition of 3,5-dinitrophenyl derivatives in cyclodextrins. They found that the proton peak of the methyl and phenyl groups was shifted to a larger chemical shift. Furthermore, there is no overlap with the proton peak of the β-cyclodextrins in this range. The α-methyl proton

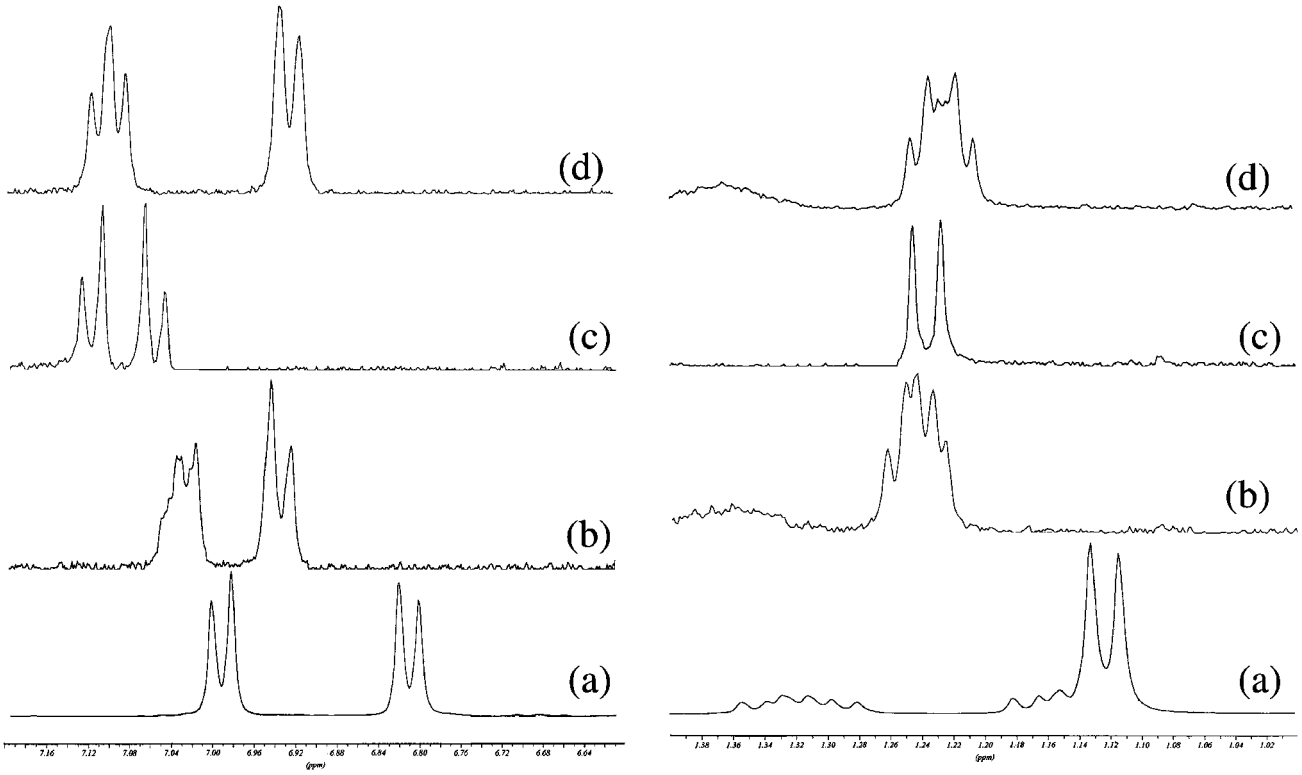

Figure 3. ^{1}H NMR spectra of loxoprofen with β-CDs in D_2O. (a) loxoprofen, (b) β-CD complex, (c) sulfated β-CD complex, and (d) glycerol ether β-CD complex.

peak of loxoprofen is in the 1.0–1.4 ppm range. In all cases, the methyl protons displayed peak shifts on complexation with cyclodextrins. In Figure 3(b), the methyl proton of the loxoprofen shifted to a larger chemical shift (ppm) due to inclusion formation, and split into two due to diastereotopic methyl resonance. However, the methyl proton of loxoprofen in Figure 3(c) also shifted to a larger chemical shift due to inclusion formation, but without any splitting due to diastereomer. In Figure 3(d), the methyl proton of loxoprofen within glycerol β-CD shifted to a larger chemical shift due to inclusion formation, and split due to diastereotopic methyl resonance. From the results of UV-VIS and ^{1}H NMR spectroscopy, the loxoprofen was inserted into the hydrophobic cavity of β-CDs in aqueous medium.

Characterization of the inclusion complexes and physical mixture

Despite the widespread use of β-CDs as solubilizers for poorly soluble drug substances, there are relatively few studies on a comprehensive investigation of β-CD/drug complexes by thermal analysis, IR, FT-Raman spectroscopy, and X-ray diffraction. When used in combination, these techniques can elucidate the host-guest interaction as well as the relation between structure and thermal decomposition. The physical mixture of the β-CD/drug for comparison with the drug/β-CD complexes was also investigated.

Figure 4 shows the TGA curves of the β-CD (a), the sulfated β-CD (b), and glycerol ether β-CD complexes (c): **1** β-CDs, **2** inclusion complex, **3** physical mixture, **4** loxoprofen. In Figure 4(a) **1**, **2**, and **3**, the first weight loss around 100 °C can be explained as the loss of moisture, and the second weight loss around 300 °C due to the decomposition of the β-CD. In the case of loxoprofen, the first weight loss occurs around 250 °C. In Figure 4(b), the weight loss of **2** around 100 °C may be considered as the loss of moisture, and the second weight loss from 210 °C to 280 °C may be due to desulfonation (SO_2), and the third weight loss around 300 °C may be due to decomposition of the glucose unit in CD. In Figure 4(c), the weight loss value for H_2O of **1**, **2**, and **3** was larger than that observed for β-CD and sulfated β-CD. These results lead us to conclude that the hydrophilicity of a glycerol ether β-CD was higher than that of the β-CD and sulfated β-CD.

Figure 5 shows the DSC curves of the β-CD (a), the sulfated β-CD (b), and glycerol ether β-CD (c): **1** β-CDs, **2** inclusion complex, **3** physical mixture, **4** loxoprofen. In Figure 5(a), the endothermic peak at 195 °C of the β-CD (**1**), and the endothermic peak at 83 °C of the loxoprofen (**4**) were observed. The endothermic peak appeared at 162 °C for the inclusion complex. In the physical mixture of loxoprofen and β-CD, the endothermic peak around 82 °C is due to loxoprofen and at 195 °C is due to β-CD. In Figure 5(b), the endothermic peak of sulfated β-CD (**1**) was observed at 180 °C. The inclusion complex (**2**) showed an exothermic peak around 170 °C, whereas the physical mixture (**3**) showed an exothermic bimodal peak around 170 °C. The exothermic peak may be considered as originating from the reaction of the sulfated β-CD and loxoprofen. In Figure 6(c), a broad endothermic peak at 190 °C of the

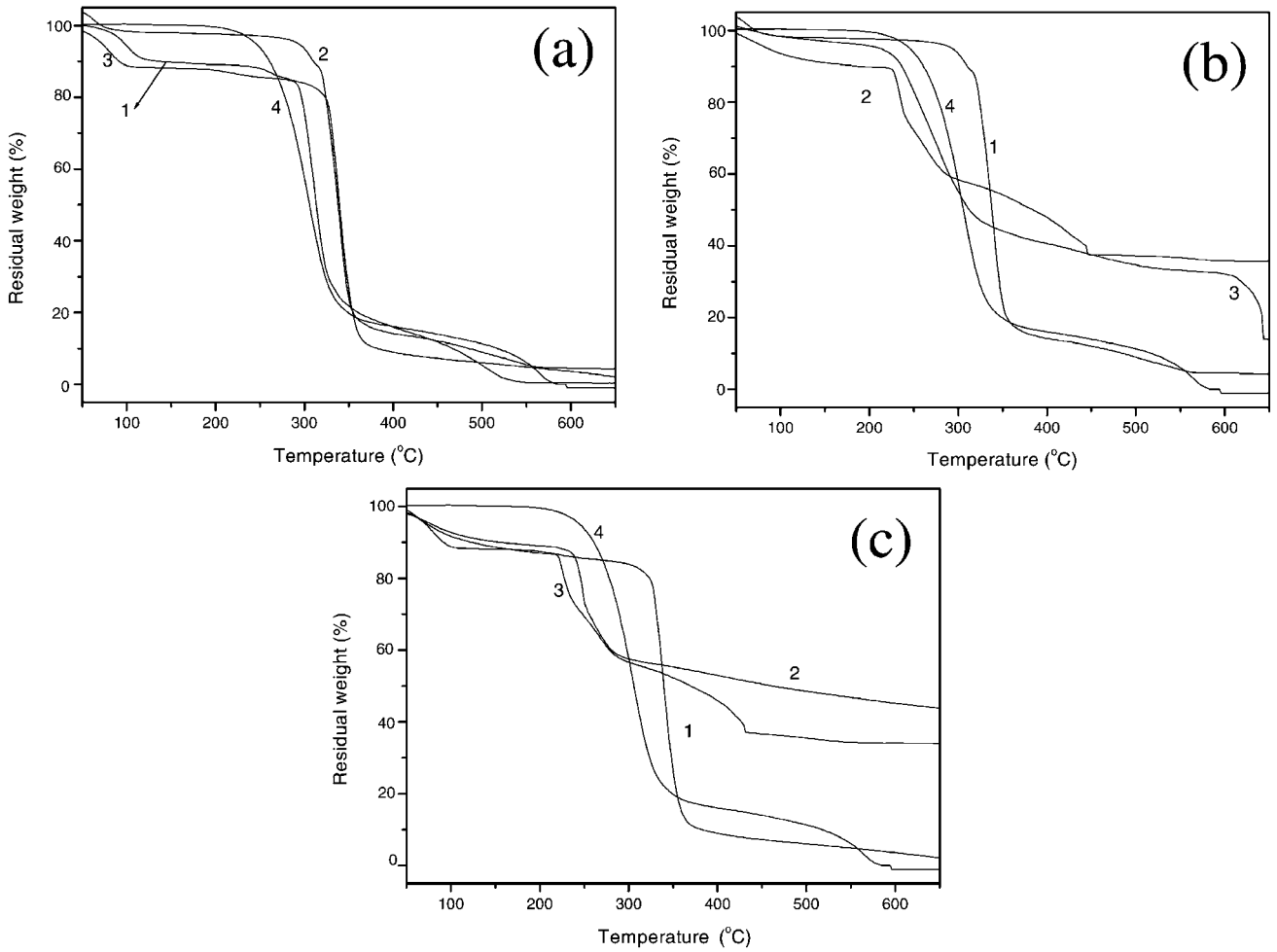

Figure 4. TGA curves of the inclusion complex and physical mixture. (a) β-CD, (b) sulfated β-CD, and (c) glycerol ether β-CD. **1** β-CD, **2** inclusion complex, **3** physical mixture, **4** loxoprofen.

glycerol ether β-CD (**1**) was observed. A very broad endothermic peak around 190 °C of the physical mixture (**3**) was observed.

In order to understand the host–guest interaction in the solid state, the inclusion complex and physical mixture were examined by IR, FT-Raman, and XRD, respectively. Figure 6 shows the FT-IR spectra of the β-CD (a), the sulfated β-CD (b), and the glycerol ether β-CD complexes (c): **1** β-CDs, **2** inclusion complex; **3** physical mixture, **4** loxoprofen. In Figure 6(a), pure loxoprofen (**4**) shows two prominent bands at 1701 cm^{-1} and 1736 cm^{-1} due to the ketone group [-C(=O)-] in the cyclic ring and the carboxylic acid group (-COOH). In Figure 6(a), the ketone vibration stretching in the physical mixture (**3**) also appeared, whereas the inclusion complex (**2**) gave very poor spectrum. In Figure 6(b), the ketone vibration stretching in the physical mixture (**3**) was decreased. In Figure 6(c), the ketone vibration stretching of the inclusion complex (**2**) again gave a very poor spectrum. These results indicated that the loxoprofen was included into the hydrophobic cavity of β-CD and glycerol ether β-CD, with possibly a different arrangement in the cavity of sulfated β-CD.

The 1550–1680 cm^{-1} regions of the FT-Raman spectra of the guest molecules contain the phenyl C=C stretching mode (νC=C). Furthermore, there is no overlap with that of the cyclodextrins in this range. Therefore, the 1550–1680 cm^{-1} region of loxoprofen included in β-CDs was used for the studies of inclusion formation.

Figure 7 shows the FT-Raman spectra of the β-CD (a), the sulfated β-CD (b), and glycerol ether β-CD (c): **1** loxoprofen, **2** inclusion complex; and **3** physical mixture. In Figure 7(a), pure loxoprofen (**1**) shows a prominent band at 1611 cm^{-1} due to the phenyl group (νC=C stretching). The full width at half maximum (FWHM) intensity of the inclusion complex was increased as compared with that of pure loxoprofen, and the inclusion complex was shifted to a longer wavenumber (a red shift) due to inclusion formation of a aromatic group of loxoprofen within the cavity of β-CD. In Figure 7 (b), the full width at half maximum (FWHM) intensity of the inclusion complex was increased as compared with that of pure loxoprofen, and the inclusion complex was shifted to a longer wavenumber (a red shift) due to inclusion formation of an aromatic group of loxoprofen within the cavity of sulfated β-CD. In Figure 7(c), the full width at half maximum (FWHM) intensity of the inclusion complex was increased as compared with that of pure loxoprofen.

Figure 8 shows the XRD spectra of the β-CD (a), the sulfated β-CD (b), and glycerol ether β-CD (c): **1** β-CDs,

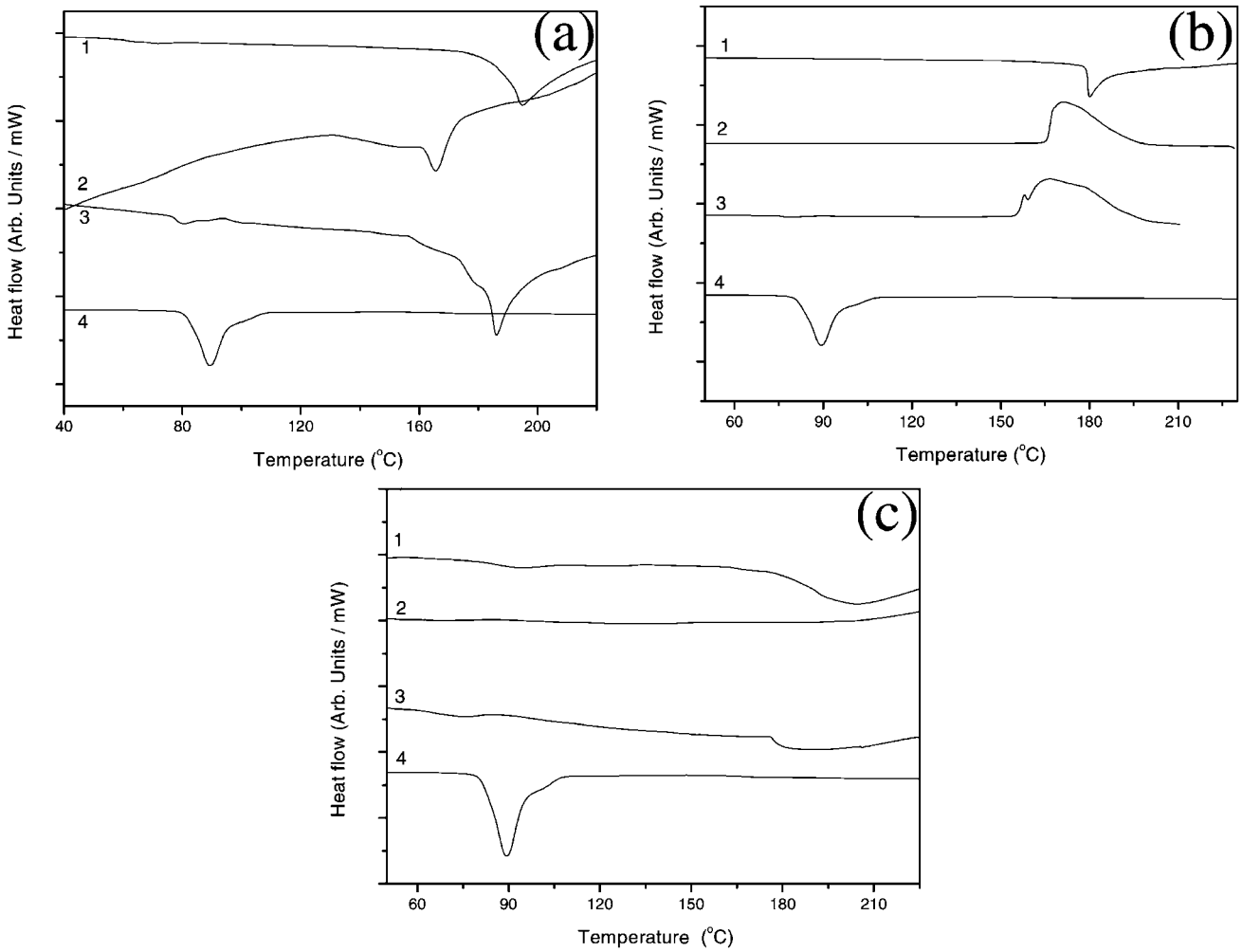

Figure 5. DSC curves of the inclusion complex and physical mixture. (a) β-CD, (b) sulfated β-CD, and (c) glycerol ether β-CD. **1** β-CD, **2** inclusion complex, **3** physical mixture, **4** loxoprofen.

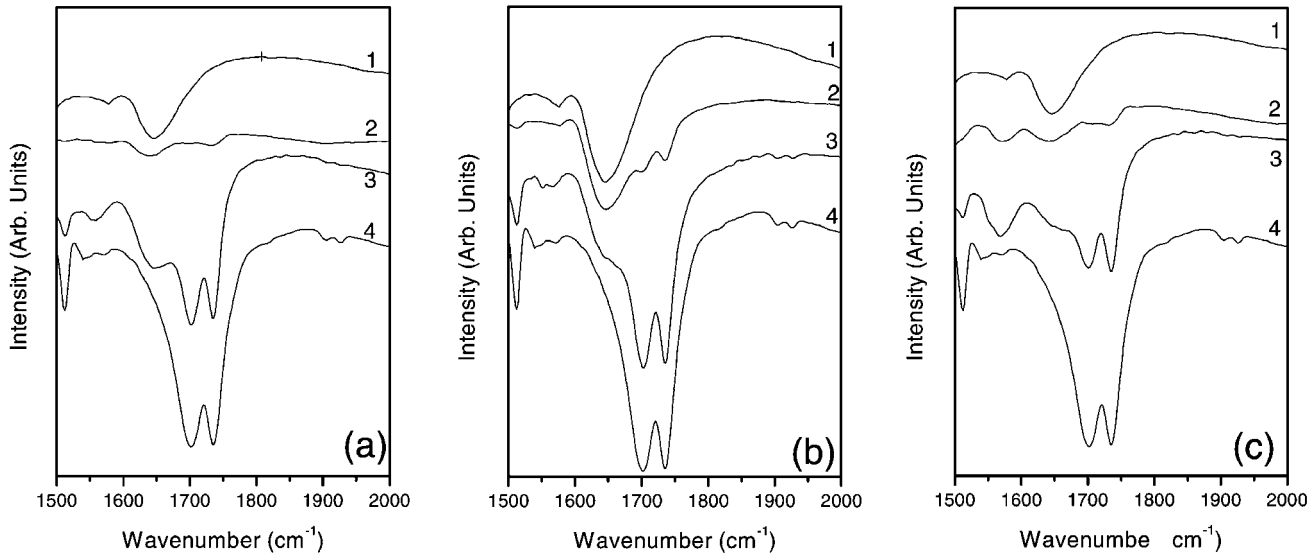

Figure 6. FT-IR spectra of loxoprofen with (a) β-CD, (b) sulfated β-CD, and (c) glycerol ether β-CD. **1** β-CD, **2** inclusion complex, **3** physical mixture, **4** loxoprofen.

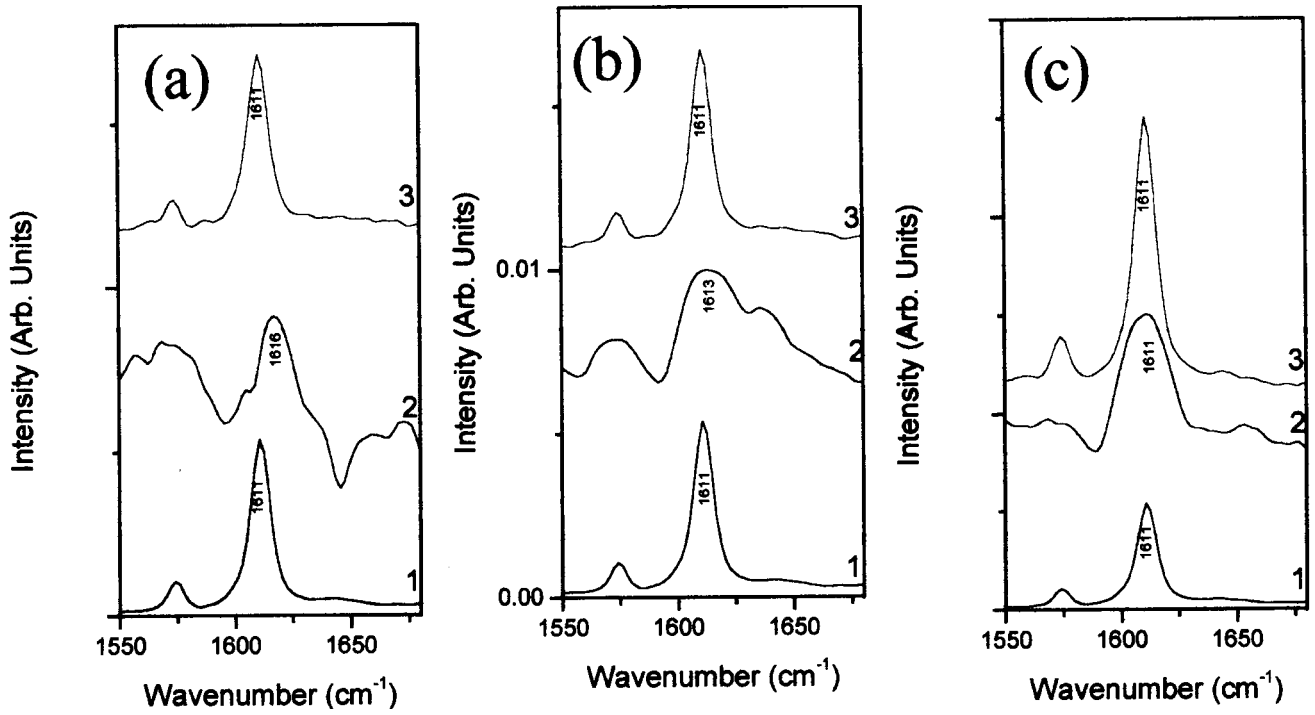

Figure 7. FT-Raman spectra of loxoprofen within β-CD, (a) sulfated β-CD (b), glycerol ether β-CD (c). **1** loxoprofen, **2** inclusion complex of loxoprofen with β-CDs, **3** physical mixture.

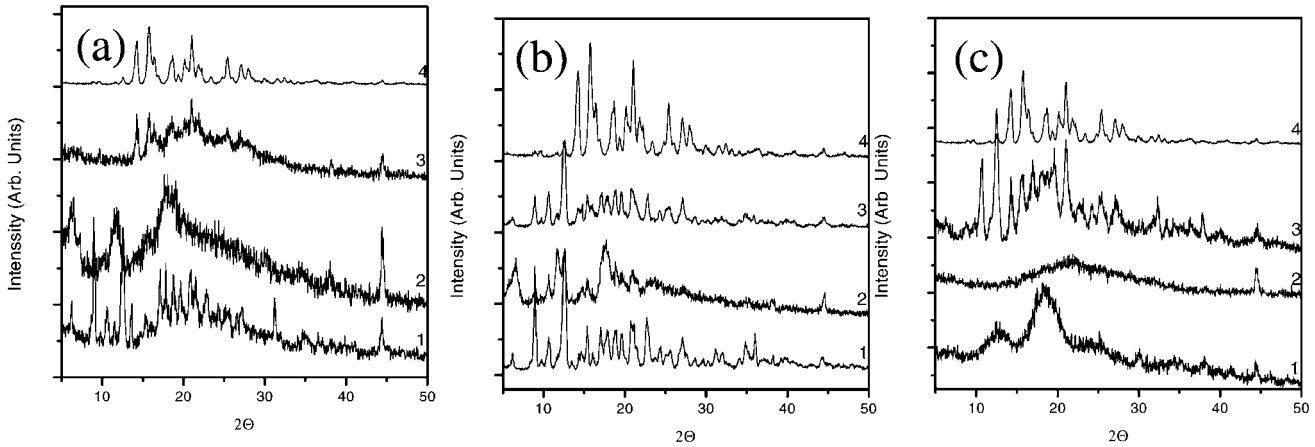

Figure 8. XRD spectra of loxoprofen with β-CD, (a) β-CD, (b) sulfated β-CD, and (c) glycerol ether β-CD. **1** β-CDs, **2** inclusion complex, **3** physical mixture, **4** loxoprofen.

2 inclusion complex, **3** physical mixture, **4** loxoprofen. The spectra of the loxoprofen/β-CD complex was clearly different from those of the physical mixture. It showed amorphous behaviour as evidenced by broader peaks of lower intensity. In the case of sulfated β-CD and glycerol β-CD, a different crystal structure was observed.

Figure 9 shows the photomicrographs of the β-CD (a), the sulfated β-CD (b), and glycerol ether β-CD (c): **1** β-CDs, **2** inclusion complex, **3** physical mixture, **4** loxoprofen. In Figure 10(a), the scanning electron microscopy (SEM) image of the physical mixture (**3**) indicated only an addition of each component, whereas the SEM image of the inclusion complex showed a single component.

From FT-IR and FT-Raman results, the phenyl group of the loxoprofen was inserted in the hydrophobic cavity of CDs.

Conclusions

(1) The solubility of loxoprofen increased with increasing β-CDs concentration, with solubility being in the following order: glycerol ether β-CD > sulfated β-CD > β-CD.
(2) The inclusion of loxoprofen within β-CDs was successfully verified by UV-VIS spectroscopy and ^{1}H NMR spectroscopy in solution.
(3) The inclusion of loxoprofen within β-CDs was successfully verified by FT-IR spectroscopy, XRD, TGA, and DSC in the solid state.

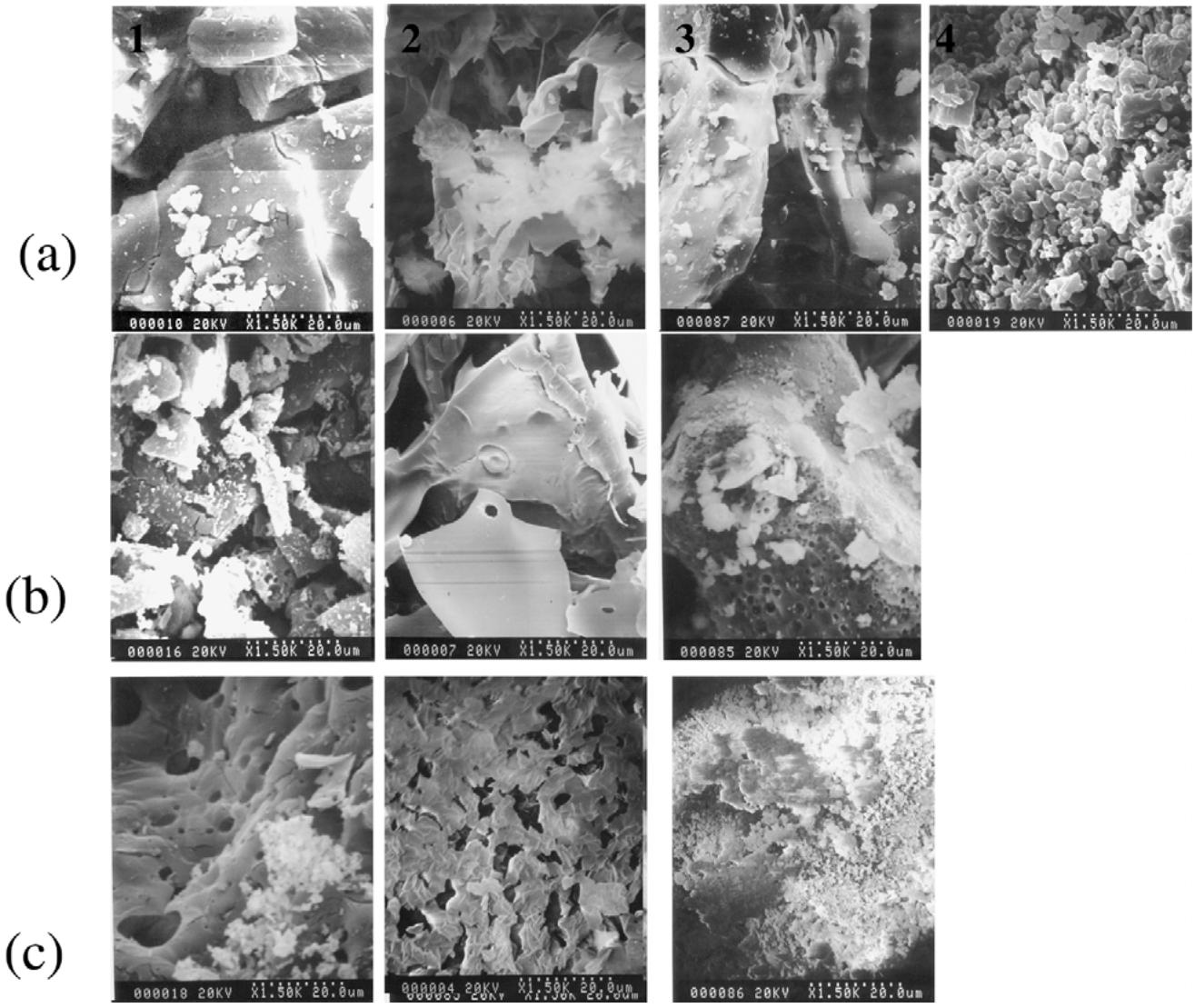

Figure 9. Surface morphology of (a) β-CD, (b) sulfated β-CD, and (c) glycerol ether β-CD. **1** β-CDs, **2** inclusion complex, **3** physical mixture, **4** loxoprofen.

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