Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **23:** 277–288, 1996.

Preparation and Characterization of the β-Cyclodextrin Inclusion Complex with Sulfafurazole

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(Received: 3 August 1995; in final form: 19 January 1996)

Abstract. The 1 : 1 inclusion complex involving sulfafurazole (SF) and β-cyclodextrin (β-CD) is prepared by the freeze-drying method and characterized on the basis of its chemical analysis, thermal behavior, infrared spectrum, X-ray powder pattern and ^{13}C NMR spectrum in DMSO-d_6 solution. The stability constant of the inclusion complex was determined by the solubility method. The effect of cyclodextrin on the UV absorption spectrum of sulfafurazole was also observed.

Key words: β-Cyclodextrin complex, sulfafurazole, freeze-drying, kneading, thermal analysis, UV spectroscopy, IR and ^{13}C NMR complexation effect.

1. Introduction

Cyclodextrins are frequently used as building blocks, because they can be linked both covalently and noncovalently with specificity [1]. Cyclodextrins are of great importance in supramolecular chemistry, since they form a homologous series of water-soluble, chiral host molecules which can be used as models for studying weak interactions.

During the last decade, cyclodextrins have aroused considerable interest in the pharmaceutical field, due to their potential to form complexes with many varieties of drug molecules [2–6]. The resulting complexes generally lead to an improvement in some of the characteristics of the guest molecules, e.g. stability, solubility and bioavailability. The complexes can be obtained in different ways, in liquid or in solid media and characterized using different methods, e.g. NMR, X-ray, IR and thermal analysis [7–11].

Various studies have been carried out on the inclusion of some sulfonamides by β-cyclodextrin in aqueous media [12–15] and in the solid state [16]. The

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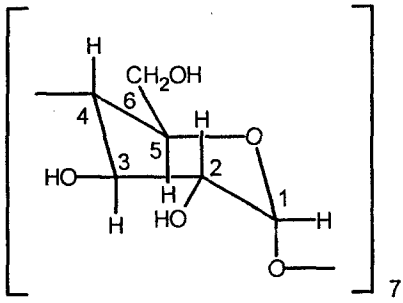
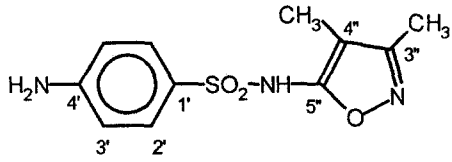
antibacterial sulfonamide, sulfafurazole (4-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-benzenosulfonamide) (SF), is constantly used in therapeutics [17, 18].



β-CD



SF

The aim of this work is to prepare a sulfafurazole–β-cyclodextrin complex, using different methods: kneading and freeze-drying. Selective physicochemical determinations based on differential scanning calorimetry, X-ray diffraction, infrared spectroscopy and carbon nuclear magnetic resonance were used to analyze this complex. The aqueous solubility improvement was also determined.

2. Experimetnal

2.1. Materials

β-Cyclodextrin (β-CD) (Chinoin, Budapest, Hungary) and sulfafurazole (SF) (Pharmaceutical Company 'Polfa', Starogard, Poland) were used as received.

2.2. Preparation of the Inclusion Complex

Kneading method: β-CD (0.178 mmol) and SF (0.178 mmol) were mixed together in a mortar; the mixture was ground for 1 h. During this process, an appropriate quantity of ethanol was added to the mixture in order to maintain a suitable consistency, Refs. [2, 19]. The paste was dried for 8 h in an oven at 70 °C and the dried complex was pulverized.

Freeze-drying method: Freeze-dried samples were prepared by dissolving β-CD and SF (molar ratio = 1 : 1) in 500 mL water and the solution was filtered. The filtrate was then frozen and lyophilized (Lyosystem I and Lyovac GT3, Leybold-Heraeus). The drug content in the freeze-dried sample was determined by UV spectroscopy; the molar ratio in the solid complex obtained was 1 : 1.

2.3. PHYSICAL MEASUREMENTS

Differential scanning calorimeter (DSC) traces were recorded on a Shimadzu DSC-50 series thermal analysis system in the range 30–480 °C at a scanning rate of 20 °C/min, under a nitrogen atmosphere; sample mass was 2 mg.

X-ray powder patterns were obtained with a TUR M62 (Germany) diffractometer with a Ni monochromator utilizing CuK_α (1.5418 Å) radiation with 30 kV and 25 mA at a scan rate of 1°/min.

FT IR spectra of samples were recorded as KBr tablets in the 4000–400 cm^{-1} region on a Bruker IFS 113v spectrophotometer.

^{13}C NMR spectra were obtained on a Varian Gemini 300VT Fourier Transform spectrometer. The chemical shifts (±0.05 ppm) were measured at 75.46 MHz. Typical conditions for the carbon spectra were pulse width 60°, FT size 64 K and digital resolution 0.6 Hz per point. Chemical shifts were measured relative to internal solvents DMSO-d_6 at 39.5 ppm, relative to TMS. The samples were prepared by dissolving the solid complex, substance alone or their physical mixture in DMSO-d_6. The concentration of sample was 0.3 M. Heteronuclear correlation experiments (HETCOR) [20] were carried out with a spectral width of ca. 3500 Hz for ^{13}C and ca. 350 Hz for ^{1}H, relaxation delay 1.013, number of increments 128 and FT size 512 × 512.

UV spectra were recorded on a Varian Cary 118C UV-VIS spectrophotometer; the measurements were carried out in Britton–Robinson buffer of pH 5.11 at the appropriate temperature. UV absorption changes of a given SF (constantly 4.16×10^{-5} M) in the presence of β-CD (varied from 3.0×10^{-3} to 9.0×10^{-3} M) were measured at the maximum wavelength due to the complex formation.

2.4. SOLUBILITY AND DISSOLUTION STUDIES

The solubility studies were carried out according to Higuchi and Connors [21]. The excess amount of SF was added to aqueous β-CD solution and shaken at 25 °C for 24 h until the system attained equilibrium. Then the solutions were filtered through Whatman 0.65 μm filter and analyzed spectrophotometrically. The presence of β-CD did not interfere with the spectrophotometrical assay. The complex formation constant was calculated from the phase diagram obtained according to the Higuchi and Connors method [21].

Determination of the dissolution rate of SF from the complex was carried out in an Erweka DZT paddle apparatus at 37 ± 0.5 °C and 150 rpm. The absorbance at 260 nm was measured using a Zeiss Jena M-40, spectrophotometer.

3. Results and Discussion

3.1. Thermal Analysis

DSC curves of SF, β-CD, SF–β-CD complexes and a physical mixture of β-CD + SF (molar ratio 1 : 1) are shown in Figure 1.

The DSC curve of β-CD shows two endotherms at 105 °C and 335 °C; the first one corresponds to the loss of H_2O, the latter to decomposition of β-CD (Figure 1b).

The sharp endothermic peak at about 199 °C and an exothermic peak at about 250 °C were observed for SF, physical mixtures and also for the product obtained by kneading. On the DSC curve of the kneading product three endothermic peaks were found. The first peak at 95 °C corresponds to the loss of H_2O, the second at 201 °C is a result of SF melting. The last peak slightly decreased, and the third one at 285 °C is due to decomposition of the β-CD molecule. The endothermic peak (at 201 °C) of SF due to the fusion of the drug does not appear in the DSC curve for the freeze-dried product (Figure 1e); it may be attributed to the amorphous state and/or inclusion complex formation.

3.2. X-Ray Powder Diffraction

The X-ray powder patterns for the individual components, complexes and physical mixture, are reported in Figure 2.

A comparison of the SF–β-CD diffraction patterns with that of the physical mixture reveals marked differences. Furthermore, the data for the physical mixture can be interpreted as an approximate superposition of the components.

One peak at an interplanar distance of 5.64 Å is evident in the freeze-dried product (Figure 2e). These results suggest that the freeze-dried SF–β-CD product may be an inclusion complex. Alternatively, the data might be explained solely in terms of the formation of the amorphous drug on freeze-drying with β-CD.

The SF–β-CD kneaded product exhibited lower crystallinity than the corresponding physical mixture, however, characteristic SF peaks (7.25, 6.66, 5.04, 3.87 and 3.31 Å) were still observed. These observations reinforce the evidence from ^{13}C NMR analysis that the kneaded product is a new phase associated with the formation of an inclusion complex.

3.3. IR Spectra

Generally IR techniques are not suitable for the detection of inclusion compounds because the resultant spectra have a superposition of host and guest bands [22, 23]. Fortunately, in this study, due to the fact that SF has some characteristic IR absorption bands in the region where β-CD does not absorb, this region could be used to detect a guest interaction.

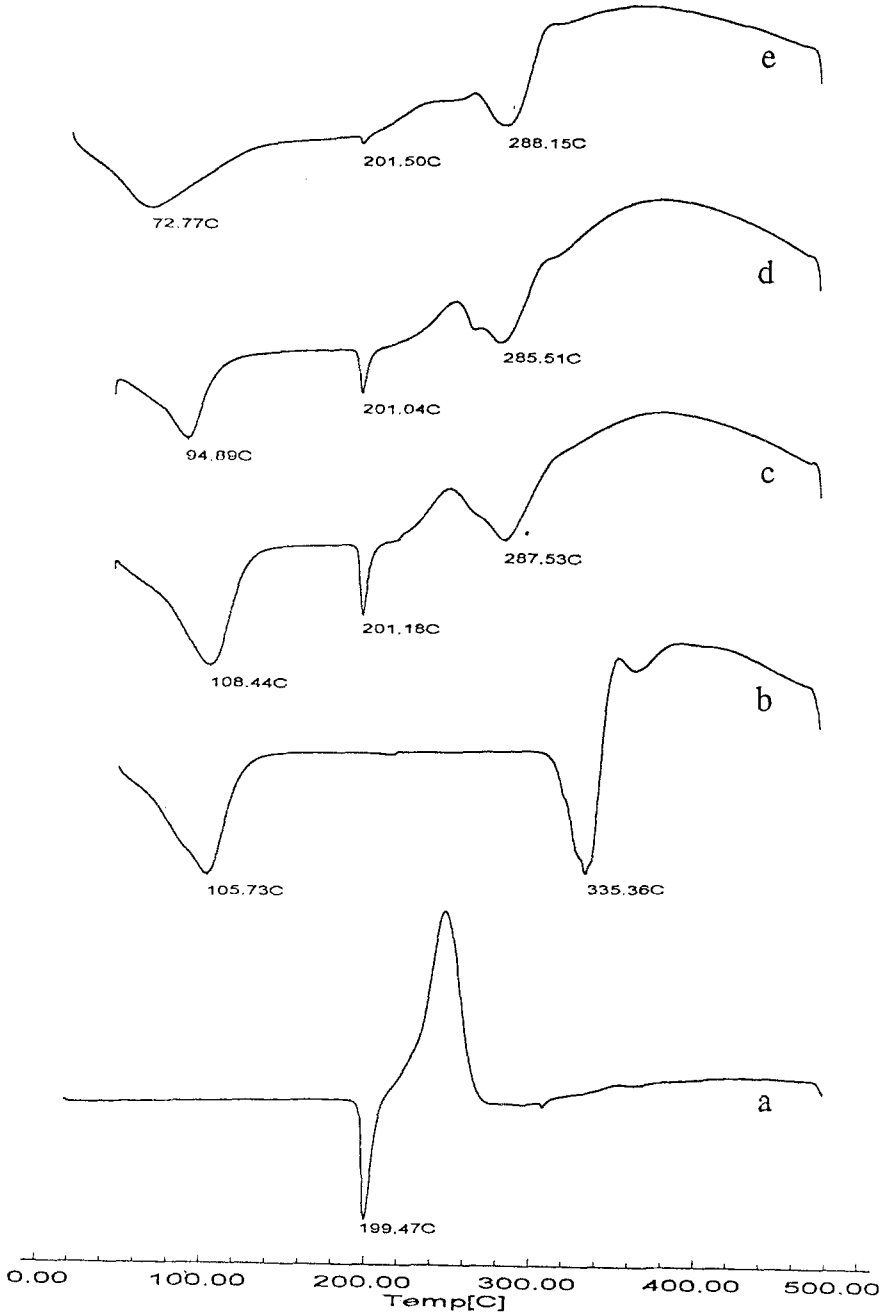


Figure 1. DSC curves of sulfafurazole and its complexes prepared by kneading and freeze-drying methods. (a) SF, (b) β-CD, (c) physical mixture β-CD + SF, (d) kneaded sample SF–β-CD, (e) freeze-dried sample SF–β-CD.

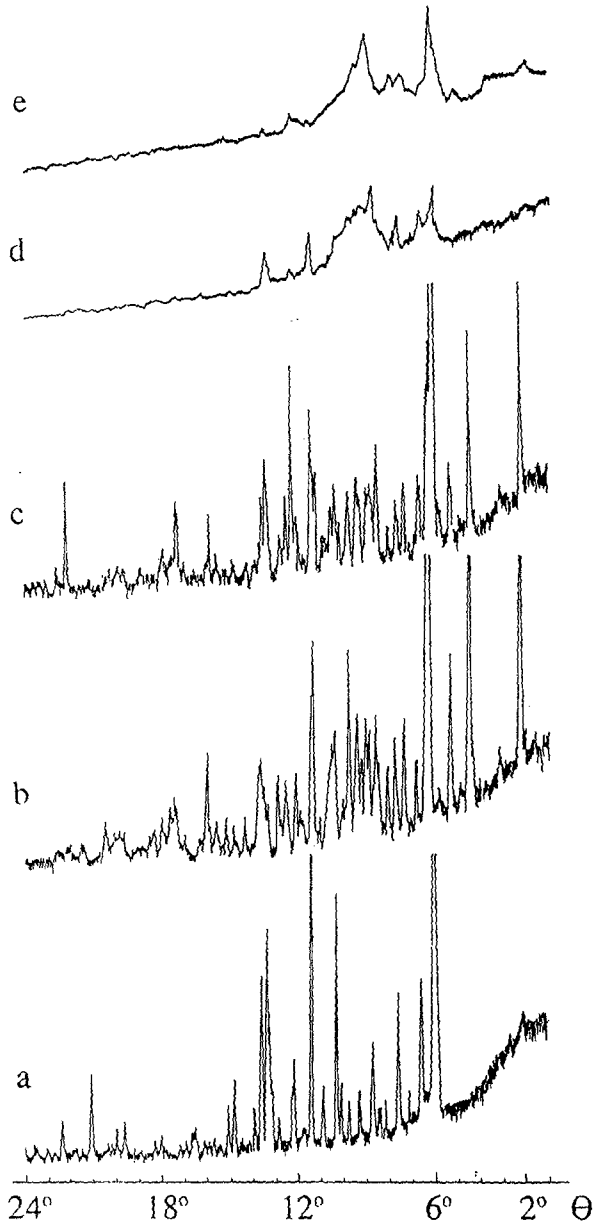


Figure 2. X-ray diffraction patterns of: (a) SF; (b) β-CD; (c) physical mixture β–CD + SF; (d) kneaded sample SF–β-CD; (e) freeze-dried sample SF–β-CD.

The IR spectra of free SF and its physical mixtures with β-CD show four well-resolved bands at 1647, 1631, 1597 and 1573 cm^{-1} due to the ring stretching modes (Figure 3a,c). In the complex obtained by freeze drying, these bands are less resolved (Figure 3e); two bands at 1648 and 1597 cm^{-1} and two shoulders at

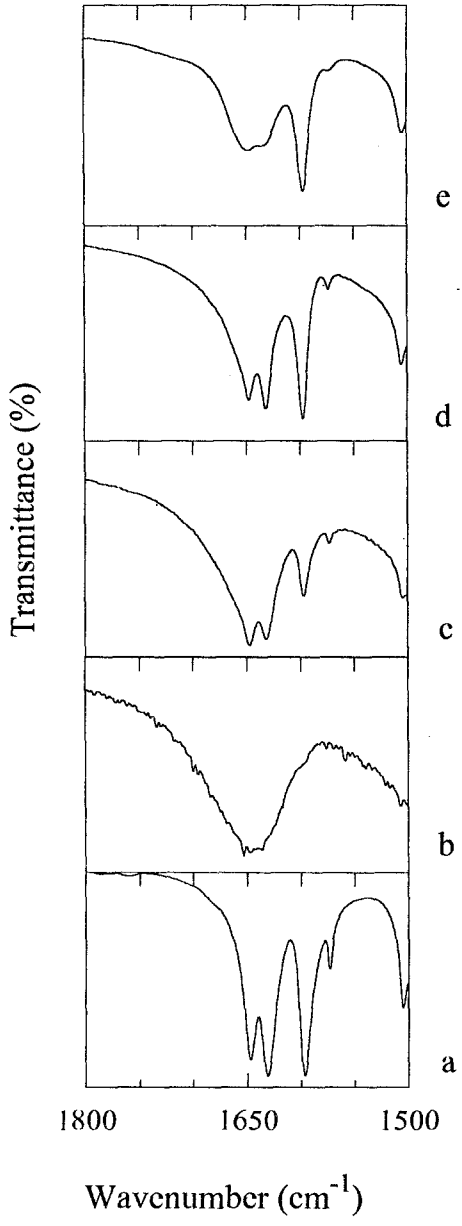


Figure 3. IR spectra (1800–1500 cm^{-1}) in KBr of: (a) SF; (b) β-CD; (c) physical mixture β-CD + SF; (d) kneaded sample SF–β-CD; (e) freeze-dried sample SF–β-CD.

1635 and 1577 cm^{-1} are observed. Thus, the observed conversion of bands into shoulders can be taken as evidence of inclusion.

The complex obtained by the kneading method did not seem to be a true inclusion compound. The IR spectra (Figure 3) did not show any difference between the main

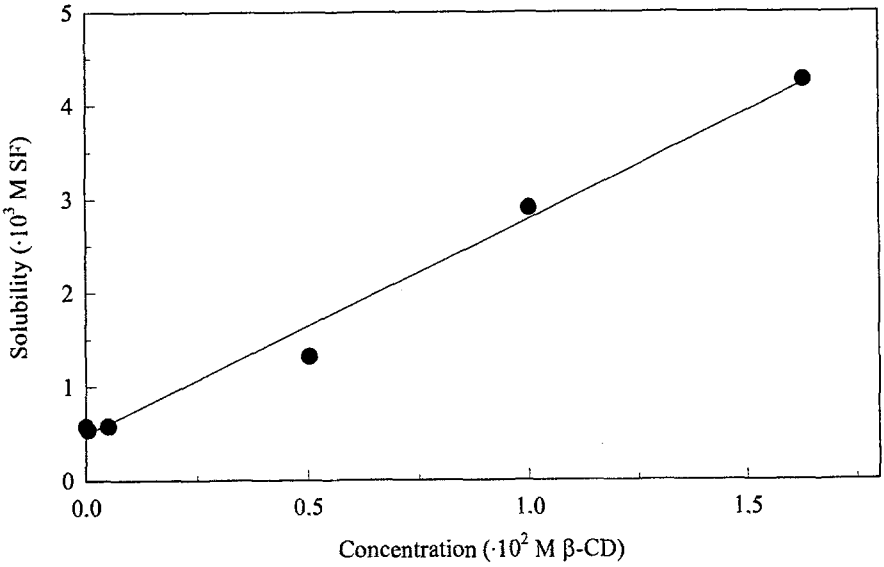


Figure 4. Apparent solubilities (25 °C) of SF in β-CD solutions.

SF absorption bands on the one hand, and the complex and physical mixture on the other.

3.4. ^{13}C NMR

The numbering of the carbon atoms of β-CD are according to Alves and Fonseca [24]. In the case of SF chemical shifts for the benzene residue were estimated from SCS data listed by Ewing [25] and for the heterocyclic residue were designated on the basis of data in Refs. [26, 27] and heteronuclear correlation experiments.

The chemical shifts for the physical mixture of β-CD with SF are very similar to the chemical shifts of the free compounds. The largest variation of chemical shifts are observed for the carbon atoms of SF in the freeze-dried complex: atoms 1′ (+0.23 ppm) and 3″ (+0.27 ppm) are deshielded while atom 4″ (−0.30 ppm) is shielded (Table I). Most chemical shifts for the kneaded complex are very close to those of the physical mixture. Atoms 1′ and 3″ show differences of +0.11 and +0.07 ppm, respectively. This suggests that in the kneaded sample the amount of the inclusion complex is low. Large variations of chemical shifts of the 3″, 4″ and 1′ carbon atoms in the freeze-dried sample indicate that these atoms are included into β-CD cavity.

3.5. Solubility and Dissolution Studies

The solubility of SF increased with the addition of β-CD in the range 0–1.629 · 10^{-2} M (Figure 4). The curve of SF solubility displays an A_L-type phase diagram, indicating that a soluble inclusion compound is formed. Assuming the formation

Table I. Chemical shifts (ppm) of β-cyclodextrin (β-CD), sulfafurazole (SF), the SF–β-CD complex in DMSO-d_6 and the physical mixture (β-CD + SF).

Carbon	β-CD/SF (δ)	β-CD + SF (δ)	SF–β-CD[a] ($\Delta\delta$)[c]	SF–β-CD[b] ($\Delta\delta$)[c]
β-CD	β-CD[d]			
1	101.96	101.89	+0.02	+0.03
2	72.46	72.41	+0.03	+0.03
3	73.14	73.05	+0.03	+0.04
4	81.59	81.53	+0.03	+0.03
5	72.12	72.02	+0.05	+0.06
6	60.03	59.95	+0.04	+0.04
SF	SF[e]			
1′	124.38	124.45	+0.11	+0.23
2′	128.58	128.58	+0.02	+0.01
3′	112.55	112.59	+0.04	+0.04
4′	153.13	153.14	0.00	−0.05
3″	156.06	156.13	+0.07	+0.27
4″	104.24	104.24	−0.04	−0.30
5″	161.02	161.07	+0.02	−0.01
CH_3 (3″)	10.39	10.44	+0.02	+0.03
CH_3 (4″)	5.92	5.95	+0.06	+0.06

[a] Kneaded complex.
[b] Freeze-dried complex.
[c] $\Delta\delta = \delta_{\text{complex}} - \delta_{\beta\text{-CD+SF}}$; + and − indicate deshielding or shielding, respectively.
[d] Chemical shifts of β-cyclodextrin.
[e] Chemical shifts of sulfafurazole.

of a 1 : 1 complex in solution, the apparent stability constant K was calculated according to Equation (1), where S is the slope of a plot of SF solubility versus β-CD concentration and C_S is the drug solubility [28].

$$K = \frac{S}{C_S \cdot (1 - S)} \tag{1}$$

The value of the stability constant was found to be 640 M^{-1}.

The results of the solubility study (μg/mL), carried out in water, show that the presence of cyclodextrins leads to an improvement in the solubility of SF (SF 249.66; physical mixture β-CD + SF 305.10; complex SF–β-CD 361.34). However, it does not seem to be the only significant difference in the improvement caused by the kneaded complex compared with that resulting from the physical mixture.

On the other hand, as can be seen in Figure 5, the SF dissolution rate from the complex is clearly increased compared with that of the physical mixture and SF

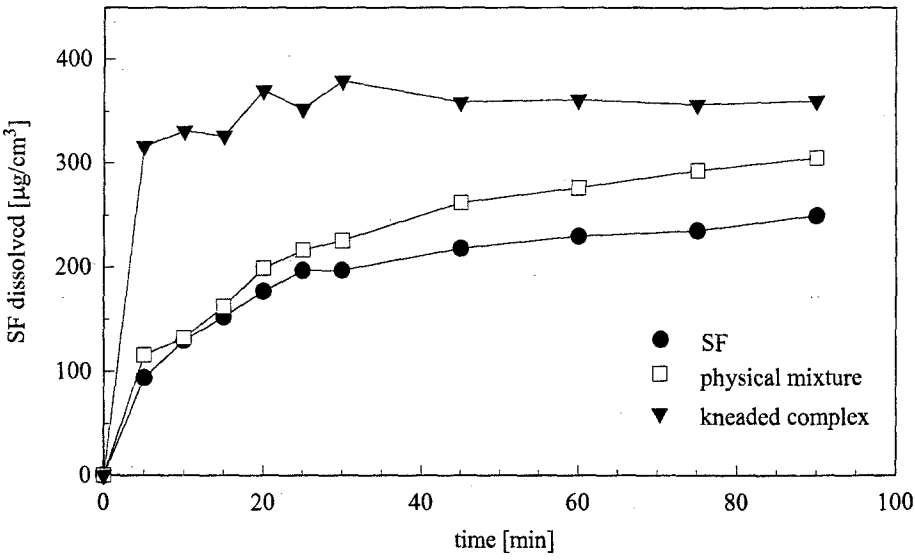


Figure 5. Dissolution profiles SF and its kneaded complex in distilled water at 50 rpm and 37 °C.

alone. At 5 min. nearly 316.34 μg/mL (86%) of SF is dissolved from the kneaded complex, compared with only 115.86 μg/mL (31%) from the physical mixture.

3.6. UV Absorption

Figure 6 shows the effect of β-CD on the UV absorption spectrum of SF, where the absorption maximum (λ_{max}) and intensity changed concomitantly with the increasing amount of β-CD. At the largest concentration of β-CD batochromic effects were observed from 253 to 262 nm. A clear isosbestic point was observed at 224 nm, but two unrelated isosbestic points appeared at 266 and 270 nm, further indicating a complicated mechanism of interaction in this system.

4. Conclusions

The DSC curves, IR spectra and powder X-ray diffraction patterns reported here indicate the formation of a stable 1 : 1 complex of SF–β-CD in the solid state for the freeze-dried sample. Using the kneading method a complex and physical mixture are formed together.

The ^{13}C NMR data for the freeze-dried sample in DMSO-d_6, solubility and UV studies in water confirm the existence of the complex in solutions.

The findings of this research testify to the fact that the inclusion complex SF–β-CD increased the aqueous solubility of the pure drug by about 45%.

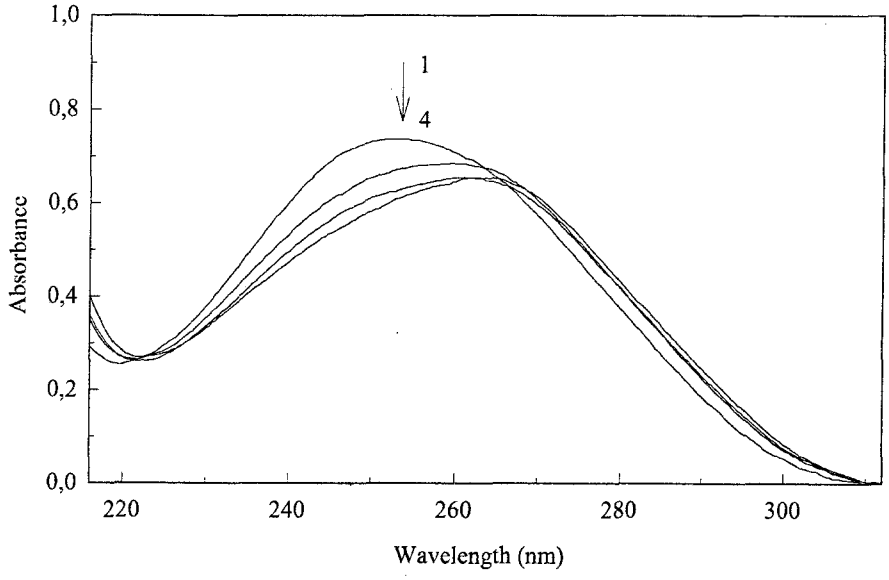


Figure 6. Effect of β-CD on the UV Absorption Spectrum of SF in Britton–Robinson Buffer (pH 5.11). Concentration of SF: constant 4.16×10^{-5}; M; concentration of β-CD added: curve (1) 0, (2) 3.0×10^{-3} M, (3) 4.5×10^{-3} M, (4) 9.0×10^{-3} M.

Acknowledgements

We are grateful to Professor Dr. B.W. Müller, Department of Pharmaceutics, Christian-Albrechts University of Kiel, for making possible the use of the lyophillizers. This work was supported by KBN (grant 2303/4/91).

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