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Solid-state β-cyclodextrin complexes containing indomethacin, ammonia and water. II. Solubility studies

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Abstract

In a previous report five unique complexes were prepared and characterized containing β-cyclodextrin, indomethacin, ammonia and water. In this study the solubility, dissolution behavior, complex-binding constant, crystallinity and enthalpy were assessed. The results show indomethacin solubility was improved by complex formation with β-cyclodextrin. There was little difference among the various complex solubilities. Indomethacin dissolution was improved by complex formation with the exception of one complex. Indomethacin dissolution profiles were found to differ and were unrelated to either the complexed indomethacin content or binding constant. Indomethacin dissolution profiles were found to be related to the complex crystallinity and enthalpy. The complex-binding constants were found to support a theory reported previously that β-cyclodextrin ring cavity solvation was the predominant factor responsible for complex formation.

Keywords: β-Cyclodextrin; Indomethacin; Solubility; Dissolution; Binding constant; Crystallinity; Enthalpy

1. Introduction

In a previous report (Casella et al., 1998) five trimolecular complexes of indomethacin, ammonia and water were prepared with β-cyclodextrin. Each complex was found to have a unique physical character. These complexes contained a different amount of complexed indomethacin in one or more of the indomethacin physical forms.

The authors theorized that complex-binding strength was controlled by the hydration of the indomethacin carboxylate anion within the β-cyclodextrin ring cavity. They reasoned this hydration was controlled by a water and ammonia network which was formed within the β-cyclodextrin ring cavity prior to indomethacin complexation (Casella et al., 1998).

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To expand on earlier findings (Casella et al., 1998) the β-cyclodextrin complexes were investigated to estimate the stoichiometry and associated binding constant (K), to assess the dissolution characteristics in capsule dosage form, and to measure the pH uncorrected solubility in simulated gastric fluid (USP, 1990) and in distilled water. The complex crystallinity and thermal behavior were investigated and compared to the dissolution characteristics. Cyclodextrins have been shown to improve the dissolution rate of poorly water-soluble drugs (Uekama et al., 1983a,b; Seo et al., 1983; Imai et al., 1988; Yu et al., 1989).

2. Experimental materials

Indomethacin was obtained from Kalipharma Pharmaceutical, Elizabeth, NJ; pharmaceutical grade β-cyclodextrin was obtained from Sanraku, Chemical Division, Tokyo, Japan; ammonium carbonate was obtained from Fisher Scientific, Medford, MA; Avicel PH101 was obtained from FMC Corporation, Newark, DE; and Indocin capsules were obtained from Merck, Sharp and Dohme, West Point, PA.

3. Complex Preparation

The complexes used in this investigation were prepared by the method reported previously (Casella et al., 1998).

4. Indomethacin determination by UV analysis

The indomethacin content was determined by a stability-indicating ultraviolet (UV) assay method at a wavelength of 318 nm (λ_{max}). Calibration standards of pure indomethacin were prepared and diluted in various aqueous ammonia solutions (see phase-solubility analysis section for rationale). The calibration curves were constructed by plotting absorbance against concentration.

5. Method validation

Because complexation with β-cyclodextrin is known to cause band-broadening and/or bathochromic shifts in the spectra of UV active compounds (Szejtli, 1982), one could postulate the UV characteristics of indomethacin could be affected by β-cyclodextrin. UV analysis would be an unsuitable analytical method if band broadening was to occur. To confirm the spectral character, the UV absorbance of a 50 mg sample of each complex was measured and compared to similar samples measured by a previously reported HPLC method (Casella et al., 1998). Both methods employed calibration plots obtained from pure indomethacin standard solutions.

6. Solubility studies

6.1. Phase-solubility analysis

The phase-solubility experiment was performed by the method reported by Higuchi and Connors (1956) and Connors (1987). Each experiment was conducted by adding the substrate indomethacin and the ligand β-cyclodextrin to an aqueous ammonia solution identical to that used to prepare each complex as reported previously (Casella et al., 1998). Thus, the β-cyclodextrin ring cavity solvation could be replicated to investigate the non-ideal solubility behavior associated with each complex.

Samples were prepared in triplicate by adding 20 ml of the appropriate ammonia solution to a series of 100 ml tubes each containing successively increasing quantities of β-cyclodextrin as follows: 0, 3, 6, 9 and 12 mM. Excess indomethacin was added into each tube to maintain saturated conditions. Each tube was capped and rotated for 4 h in a constant temperature water bath at 25 ± 1°C. Following equilibrium each supernatant phase was removed, filtered, diluted and assayed for the total dissolved indomethacin content by UV analysis. The phase-solubility diagram was constructed by plotting the total dissolved indomethacin concentration against the total β-cyclodextrin concentration. The binding constant

($K_{1:1}$) was calculated as follows from the phase-solubility slope, where S_o is the solubility of indomethacin in the absence of β-cyclodextrin:

$$K_{1:1} = \frac{\text{Slope}}{S_o(1 - \text{Slope})} \quad (1)$$

6.2. pH Uncorrected solubility

The pH uncorrected solubility of indomethacin was determined in simulated gastric fluid without enzymes and in distilled water. Samples were prepared in triplicate by adding 10 ml of test fluid and excess solid powder into 100 ml glass tubes which were capped and rotated in a constant temperature water bath at 37.5 ± 1°C. Samples were collected after 1–3 h and assayed quantitatively for indomethacin and qualitatively for its two hydrolytic degradation products by a previously reported HPLC method (Casella et al., 1998). Equilibrium solubility could not be determined due to rapid indomethacin decomposition.

6.3. Capsule dosage form preparation

All complexes and the physical mixtures were filled into a capsule dosage form by using the fraction of complex and excipient that passed through a 200 mesh sieve and was retained on a 460 mesh sieve. This fraction represented an arithmetic mean particle size of 6.70×10^{-2} mm. The pure complexes, an indomethacin (20 mg) mixture with β-cyclodextrin, and an indomethacin (20 mg) mixture with Avicel PH102^{R} were filled into no. 4 gelatin capsules.

6.4. Dissolution analysis

Dissolution analysis was performed according to the USP XXI method for each prepared formulation and Indocin. Dissolution samples were collected at 5, 10, 15 and 20 min, filtered, and UV absorbance measurements were taken. The dissolution profiles were constructed by plotting the cumulative percent drug released against time.

6.5. X-ray diffraction analysis

Crystallinity measurements of indomethacin, β-cyclodextrin, and each complex were determined from the powder X-ray diffraction spectra obtained by a previously reported method (Casella et al., 1998). The crystallinity (crystalline/amorphous ratio) was determined in triplicate based on the crystalline and amorphous area measurements. These regions were resolved according to the criteria reported by Murthy (1982), Black and Loverling (1977), and Statton (1967). The area measurements were calculated by using the software program Sigma Scan (Jandel Scientific, Corte Madera, CA). The multicomponent mass-absorption coefficient of each complex and β-cyclodextrin (hydrated) was calculated by determining the weighted fraction of the mass-absorption coefficient of β-cyclodextrin, indomethacin, water and ammonia, and determining the fraction of each element (C, H, O, N and Cl) for each component as follows:

$$u = w_1(u/p)_1 + w_2(u/p)_2 + \cdots \quad (2)$$

where: w_1 and w_2 are the weighted fractions for each element, u is the linear mass-absorption coefficient and p is the bulk density. The transmitted beam intensity (I_x) after passing through a sample thickness of x, was calculated by:

$$I_x = I_o\, e^{-(u/p)px} \quad (3)$$

where: I_o is the intensity of the incident X-ray beam measured as the crystalline or amorphous area (Cullity, 1978). The total crystallinity was calculated by:

$$\frac{I_x \text{ crystalline}}{I_x \text{ amorphous}} \quad (4)$$

6.6. Differential scanning calorimetry analysis

Differential scanning calorimetry analysis was performed by a method reported previously (Casella et al., 1998). The transitional enthalpy (endothermic) was calculated by DUPONT DSC Interactive Analysis software (v. 3.0) by integrating the area under the curve for the heat capacity. The total enthalpy was calculated by taking the

Table 1
Phase solubility data[a] and the calculated $K_{1:1}$ binding constants

Cyclodextrin (mM)	Indomethacin dissolved (mg/ml)				
	Complex A	Complex B	Complex C	Complex D	Complex E
0	2.43 ± 0.02	5.86 ± 0.11	7.33 ± 0.03	9.94 ± 0.08	11.95 ± 0.23
3	2.59 ± 0.02	6.09 ± 0.02	7.41 ± 0.15	10.23 ± 0.05	11.47 ± 0.26
6	2.82 ± 0.02	6.29 ± 0.04	8.78 ± 0.05	9.89 ± 0.18	11.50 ± 0.19
9	3.06 ± 0.02	6.65 ± 0.10	9.21 ± 0.11	10.23 ± 0.09	11.36 ± 0.04
12	3.32 ± 0.03	6.92 ± 0.06	9.32 ± 0.07	10.32 ± 0.25	11.23 ± 0.29
$K_{1:1}$ (m^{-1})	1966	986	534	128	−189[b]

[a] Mean ± S.D. of three determinations.
[b] It cannot be determined accurately, see text.

sum of the enthalpies in the temperature range of 25–250°C. At temperatures above 250°C exothermic decomposition was observed; thus, no relative information could be obtained.

7. Results and discussion

7.1. Phase-solubility analysis

Phase-solubility measurements for each complex are reported in Table 1. The phase-solubility profiles (Fig. 1) show that complexes A through to D are all class-A1 diagrams and are first-order complexes as determined by the criteria of Higuchi and Connors (1956) and Connors (1987). The binding constants are reported in Table 1.

Complex A through D binding constants decreased as a function of indomethacin (Fig. 2) and ammonia content (Fig. 3). Conversely, the binding constant increased as a function of the water content (Fig. 4). These findings support the previously reported theory stating the β-cyclodextrin ring cavity solvation was the predominant factor responsible for complex formation (Casella et al., 1998).

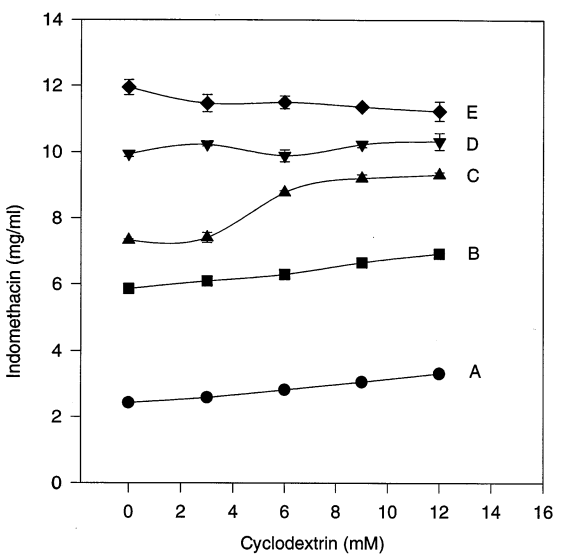

Fig. 1. Phase solubility analysis of complexes A–E.

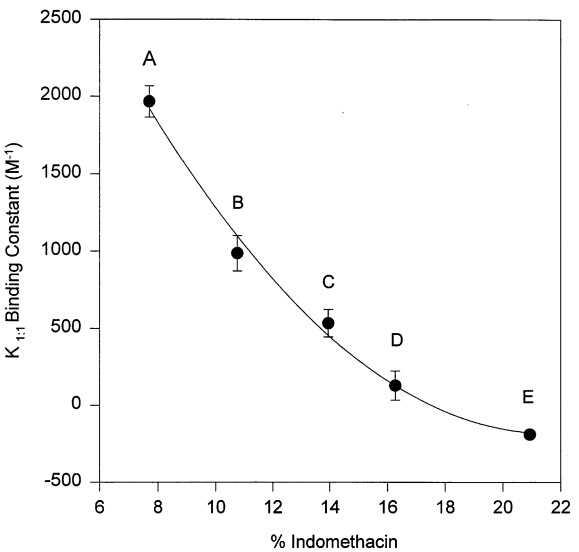

Fig. 2. The binding constant against the indomethacin content.

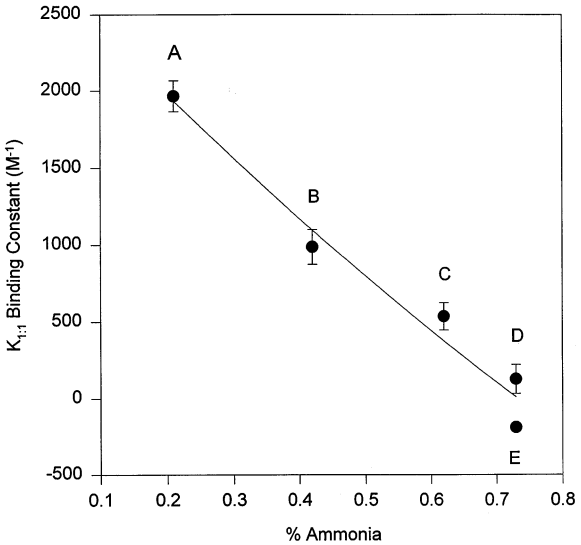

Fig. 3. The binding constant against the ammonia content.

Complex E (Fig. 1) atypically exhibits no increase in substrate solubility with an increasing β-cyclodextrin concentration. Typically, one would infer the absence of complex formation. However, the physical characterization data (Casella et al., 1998) shows a complex had formed. Because the first-order regression statistics (Fig. 1) gave rise to a negative slope the binding constant became incorrect. Thus, either a weak complex was formed and the binding constant could not be measured accurately, or this curve represented a type-B system plateau region (Higuchi and Connors, 1956; Connors, 1987).

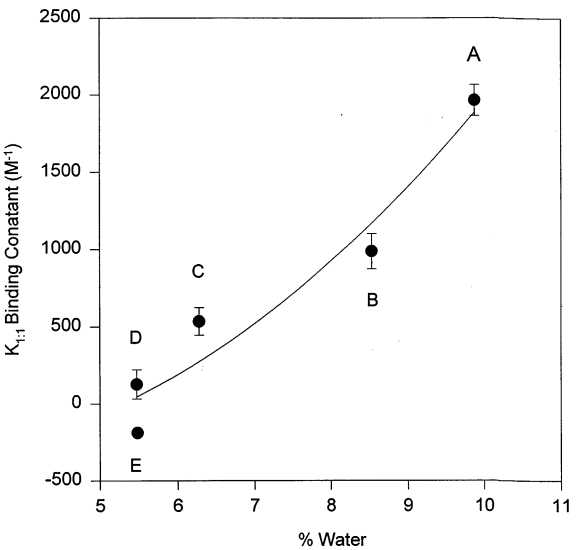

Fig. 4. The binding constant against the water content.

Table 2
The solubility of indomethacin and it β-cyclodextrin complexes in distilled water (mg/ml)[a]

Sample	0.5 h	3.0 h
Indomethacin	0.015 ± 1.0E-3	0.015 ± 2.8E-4
Complex A	1.16 ± 0.07	1.19 ± 0.17
Complex B	2.00 ± 0.11	2.10 ± 0.08
Complex C	2.55 ± 0.40	2.54 ± 0.21
Complex D	2.86 ± 0.27	3.59 ± 0.15
Complex E	3.48 ± 0.17	3.64 ± 0.26

[a] Mean ± S.D. of three determinations.

7.2. pH Uncorrected solubility

Uncorrected pH solubility measurements in distilled water and simulated gastric fluid are reported in Tables 2 and 3, respectively. Indomethacin solubility in complex form was found to be higher than pure indomethacin in distilled water (Table 2) and in simulated gastric fluid (Table 3).

Chromatograms from simulated gastric fluid samples for complexes B through E revealed the two hydrolytic degradation products of indomethacin. Since β-cyclodextrin can hydrolyze in the presence of strong acid (Szejtli, 1982), it was postulated that these complexes had dissolved in gastric fluid and subsequently decomposed.

Table 3
The solubility of indomethacin and its β-cyclodextrin complexes in simulated gastric fluid (μg/ml)[a]

Sample	0.5 h	3.0 h
Indomethacin	1.37 ± 0.06	2.10 ± 0.41
Complex A	41.27 ± 4.17	39.39 ± 5.00
Complex B	46.10 ± 6.56	37.30 ± 3.12
Complex C	40.88 ± 1.60	35.62 ± 2.02
Complex D	29.78 ± 2.44	28.43 ± 0.46
Complex E	36.49 ± 5.61	30.57 ± 1.18

[a] Mean ± S.D. of three determinations.

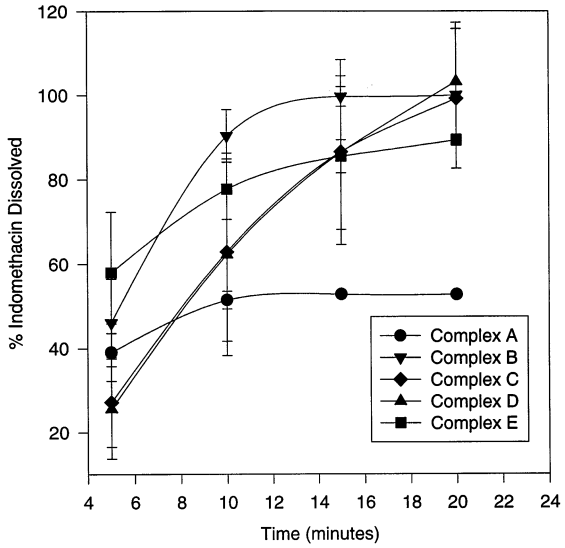

Fig. 5. Dissolution profiles of complexes A–E.

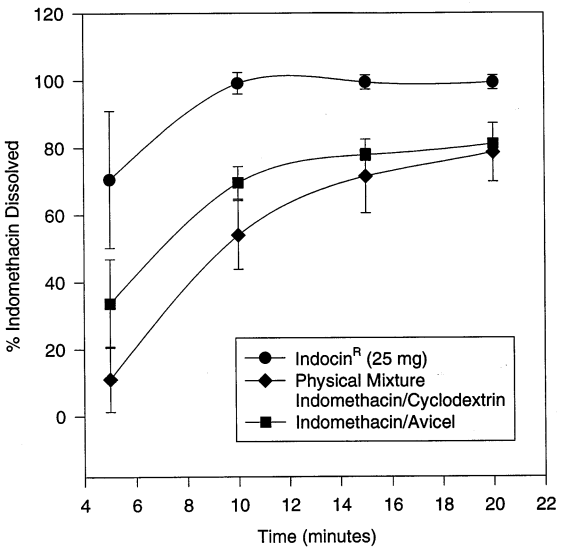

Fig. 6. Dissolution profiles of Indocin^{R} and physical mixtures of indomethacin with cyclodextrin and avicel PH102.

Therefore, these complexes would decompose if administered orally. As an exception the indomethacin in complex A was found to be stable in gastric fluid.

7.3. Dissolution analysis

The dissolution profiles of indomethacin complexes are show in Fig. 5, and dissolution profiles of Indocin and indomethacin physical mixtures are shown in Fig. 6. Indomethacin dissolution was enhanced by complexation with β-cyclodextrin with the exception of complex A. The complex B dissolution profile was found to be better than all other complexes and similar to that of Indocin. The complex A dissolution profile was inferior to all other complexes. No relationships were observed between complexed indomethacin dissolution and either the binding constants or physical/polymorphic forms present (Casella et al., 1998).

7.4. X-ray diffraction analysis to determine crystallinity

The complex crystallinity measurements are reported in Table 4. The amount of indomethacin dissolved at 10 (not shown) and 15 min (Fig. 7) was related linearly to crystallinity. The 10 and 15 min time intervals were found to discriminate among the various formulations. These findings agree with the accepted principle that an amorphous material will have better dissolution characteristics when compared to its crystalline form (Lachman et al., 1986).

7.5. Differential scanning calorimetry analysis to determine enthalpy

Complex enthalpy measurements are reported in Table 4. A relationship was discovered between enthalpy measurements and the total dissolved

Table 4
The crystallinity[a] and enthalpy associated with each complex and β-cyclodextrin

Sample	Crystallinity	Enthalpy (J/g)
Complex A	0.126 ± 0.008	291.1
Complex B	0.081 ± 0.004	228.1
Complex C	0.115 ± 0.047	211.1
Complex D	0.083 ± 0.003	178.0
Complex E	0.104 ± 0.012	244.0
β-cyclodextrin	0.175 ± 0.010	198.5

[a] Mean $\pm$ S.D. of three determinations.

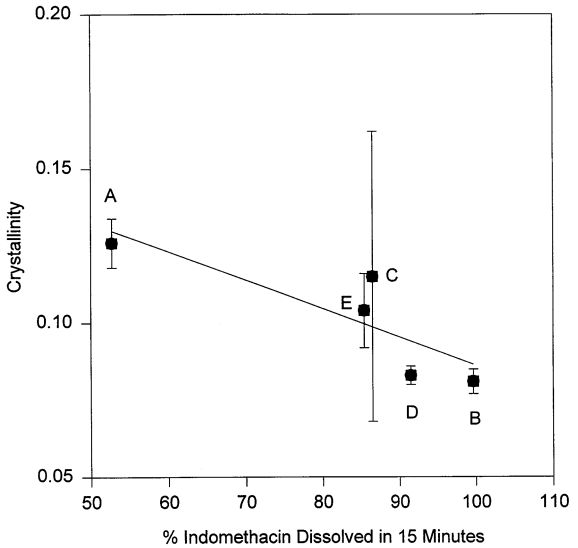

Fig. 7. The relationship between crystallinity and dissolution.

indomethacin content as shown in Fig. 8. Those complexes with a total enthalpy of less than 240 J/g were found to dissolve completely; whereas, those with an enthalpy greater than 240 J/g had incomplete dissolution in 20 min. These results explain the inferior dissolution of complex A. Hence, enthalpy measurements were found to be a suitable means for predicting the in vitro dissolution.

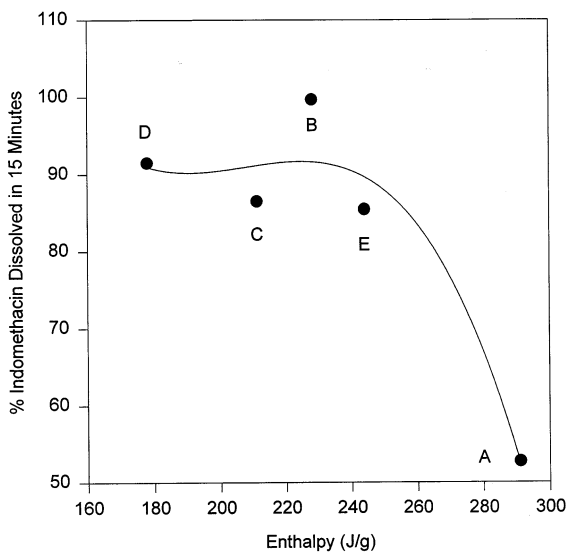

Fig. 8. The relationship between dissolved indomethacin and enthalpy.

8. Conclusions

The calculated binding constants for the five investigated complexes were found to support the previously reported theory that β-cyclodextrin ring cavity hydration was the predominant factor responsible for complex formation (Casella et al., 1998).

Indomethacin solubility was improved by complex formation. However, there was little difference among the solubilities of the five complexes.

Indomethacin dissolution in vitro was improved by complex formation with the exception of complex A. The dissolution profiles were found to differ and to be unrelated to either the complexed indomethacin content or binding constant. However, the total drug dissolved among the various complexes could be predicted by crystallinity and enthalpy measurements.

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References

Black, D.B., Loverling, E.G., 1977. Estimation of the degree of crystallinity in digoxin by X-ray and infrared methods. J. Pharm. Pharmacol. 29, 684–687.

Casella, R., Jambhekar, S.S., and Williams, D.A., 1998. Solid state β-cyclodextrin complexes containing indomethacin, ammonia and water. I. Formation studies. Int. J. Pharm. 165, 1–14.

Connors, K.A., 1987. Binding Constants. Wiley, New York, pp. 261–281.

Cullity, B.D., 1978. Elements of X-ray Diffraction, 2nd ed. Addison-Wesley, Reading, MA, pp. 13–15.

Higuchi, T., Connors, K.A., 1956. Phase solubility techniques. Adv. Anal. Chem. Instrum. 4, 117–212.

Imai, T., Otagiri, M., Saito, H., Uekama, K., 1988. Inclusion mode of flurbiprofen with β-cyclodextrin and heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin and improvements of some pharmaceutical properties of flurbiprofen by complexation. Chem. Pharm. Bull. 36 (1), 354–359.

Lachman, L., Lieberman, H.A., and Kanig, J.L., 1986. The Theory and Practice of Industrial Pharmacy, 3rd ed. Lea and Febiger, Philadelphia, PA, pp. 176–178.

Murthy, N.S., 1982. Analysis of X-ray diffraction data from polymers by profile fitting. Am. Lab. 14 (11), 70–75.

Seo, H., Tsuruoka, M., Hashimoto, T., Fujinaga, T., Otagiri, M., Uekama, K., 1983. Enhancement of the oral bioavailability of spironolactone by β- and γ-cyclodextrin complexations. Chem. Pharm. Bull. 31 (1), 286–291.

Statton, W.O., 1967. The meaning of crystallinity when judged by X-rays. J. Poly. Sci. 18, 33–50.

Szejtli, J., 1982. Cyclodextrins and their Inclusion Complexes. Akadémiai Kiadó, Budapest, pp. 38–40.

Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Yamasaki, M., Seo, H., Hashimoto, T., Tsurnoka, M., 1983a. Improvement of the oral bioavailability of digitalis glycosides by cyclodextrin complexation. J. Pharm. Sci. 72 (11), 1338–1341.

Uekama, K., Narisawa, S., Hirayama, F., Otagiri, M., 1983b. Improvement of dissolution and absorption characteristics of benzodiazepines by cyclodextrin complexation. Int. J. Pharm. 16, 327–338.

United States Pharmacopoeia, 1990. Twenty-second revision/NF, 17th ed. United States Pharmacopoeial Convention, Rockville, MD.

Yu, C.D., Sweetana, S.A., Chu, N.I., Lee, G.J.L., Massey, I.J., 1989. Enhancement of the solubility, dissolution rate and oral bioavailability of RS-82856 by complex formation with cyclodextrins. Drug Dev. Ind. Pharm. 15 (4), 609–620.

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