



The physicochemical characteristics and bioavailability of indomethacin from β -cyclodextrin, hydroxyethyl- β -cyclodextrin, and hydroxypropyl- β -cyclodextrin complexes

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Abstract

In an effort to improve the bioavailability of the insoluble drug indomethacin, three complexes were prepared with indomethacin and the soluble complexing agents β -, hydroxyethyl- β -, and hydroxypropyl- β -cyclodextrin. The indomethacin content was similar among the complexes ($P \leq 0.05$). To confirm complex formation, each complex was characterized by ultraviolet, infrared, nuclear-magnetic resonance, powder X-ray diffraction, and differential-scanning calorimetry techniques. Powder diffraction studies show the β -cyclodextrin complex was polycrystalline, and the hydroxyethyl- and hydroxypropyl- β -cyclodextrin complexes were amorphous. Phase-solubility analysis confirmed the formation of complexes and suggested the three complexes were bound similarly. Solubility studies show complexation increased indomethacin solubility, and the hydroxyethyl- and hydroxypropyl- β -cyclodextrin complexes were more soluble than the β -cyclodextrin complex in 0.1N hydrochloric acid and distilled water. Dosage forms were prepared by encapsulating the complexes without the addition of excipients. Dissolution studies show the encapsulated β - and hydroxyethyl- β -cyclodextrin complexes had superior dissolution when compared to the hydroxypropyl- β -cyclodextrin and Indocin® (50 mg) capsules. Bioavailability studies were performed by administering the indomethacin complex or Indocin capsules to male-albino, New Zealand rabbits. Indomethacin plasma-time concentration data fit best to a compartment-independent model for all capsule formulations. Bioavailability comparisons by ANOVA show no significant difference ($P \leq 0.10$) in the peak-plasma time and peak concentration among the capsule formulations. The area-under-the-curve for the β -cyclodextrin complex capsules was found to be significantly higher ($P \leq 0.10$) than all other capsule formulations. In conclusion, the bioavailability of indomethacin was improved by complexation with only β -cyclodextrin. No correlations were found among the bioavailability, solubility, and dissolution results.

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1. Introduction

The authors postulated that complex formation with either β -, hydroxyethyl- β -, or hydroxypropyl- β -cyclodextrin could improve the water solubility,

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Table 1
Characteristics of three cyclodextrins and their complexes with indomethacin

Cyclodextrin	Nominal- substitution level	M_w	Complex		
			Indomethacin ^a (%)	Water (%)	Ammonia (%)
β -Cyclodextrin	0.0	1135	10.75 \pm 0.66	8.53	0.41
Hydroxyethyl- β -cyclodextrin	3.6	1300	8.75 \pm 0.87	2.21	0.52
Hydroxypropyl- β -cyclodextrin	3.0	1330	9.30 \pm 0.62	1.58	2.27

^a Mean \pm S.D. of three determinations.

dissolution, and absorption characteristics of indomethacin (insoluble) considering the advantageous water solubility of β -cyclodextrin (\sim 2% w/v) and its hydroxyethyl and hydroxypropyl derivatives (\sim 45% w/v). This investigation was conducted to prepare inclusion compounds with cyclodextrins and the insoluble drug indomethacin, to confirm complex formation using a variety of analytical techniques, to characterize the dissolution and solubility characteristics, and to compare the bioavailability of simple capsule formulations among pure indomethacin and the prepared complexes. Other investigators have found improvements in the pharmaceutical properties of other drugs when complexes were formed with cyclodextrins (Frank, 1975; Iwaoku et al., 1982; Otagiri et al., 1983; Pitha et al., 1986; Seo et al., 1983; Uekama et al., 1979, 1983a,b). The hydroxyethyl and hydroxypropyl derivatives varied in their nominal-substitution pattern so their molecular weight increased with derivative-chain length as reported in Table 1.

Indomethacin complexes of β -, hydroxyethyl- β -, and hydroxypropyl- β -cyclodextrin were prepared in ammonia solution using a technique reported earlier (Casella et al., 1998a). Formation of complexes was confirmed using ultraviolet, infrared, nuclear-magnetic resonance, powder X-ray diffraction, differential-scanning calorimetry, and phase-solubility techniques. The pharmaceutical properties were characterized by measuring the solubility and dissolution performance. Bioavailability studies were performed by administering indomethacin to adult, male-albino New Zealand rabbits in the form of either an encapsulated cyclodextrin complex or Indocin[®] capsules. Indocin capsules were selected as a control formulation for pure indomethacin. Bioavailability comparisons were performed using pharmacokinetic data obtained from a compartment-independent model.

2. Experimental

2.1. Materials

Indomethacin, USP (polymorphic form I) was obtained from Kalipharma Pharmaceutical Co., Elizabeth, NJ, USA. Pharmaceutical grade cyclodextrins were obtained from Cerestar USA, Inc., Hammond, IN, USA. Ammonium carbonate was obtained from Fisher Scientific Co., Medford, MA, USA. Indocin capsules (indomethacin, 50 mg) were manufactured by Merck and Co., Inc., West Point, PA, USA. The Pet Piller[®] was manufactured by H-BAR-S Manufacturing, Boerne, TX, USA.

2.2. Complex preparation

The indomethacin-cyclodextrin complexes were prepared as follows (Casella et al., 1998a): 16 g of cyclodextrin were dissolved in aqueous solutions containing 4.8×10^{-2} M ammonia. Each solution was heated to 57 ± 1 °C to dissolve the cyclodextrin. Once the cyclodextrin was dissolved completely an excess of indomethacin was added to the solutions. All dispersions were stirred at 20 rpm for four hours, filtered, and dried approximately 12 h in a hot-air oven at 70 ± 1 °C, which removed ammonium carbonate (decomposes on heating) and water yielding the complexes. Each complex was an opaque glaze that varied in color from light to brownish yellow. Each complex was powdered with a mortar and pestle and stored at room temperature in light resistant containers.

2.3. Analytical methods

2.3.1. Indomethacin, ammonia, and water assay

A high performance liquid chromatographic (HPLC) assay method was developed to measure

quantitatively indomethacin and its two hydrolytic degradation products. The column (250 mm × 4.6 mm) was obtained from Alltech Associates and contained RP18 stationary phase with a mean particle size of 5 μm. Mobile-phase consisted of 65% acetonitrile and a 35% aqueous phase containing 50 mM triethylamine adjusted to pH 3.3 with phosphoric acid. The pump flow rate and the injection volume were 1.5 ml/min and 20 μl, respectively. Peak-area quantitation was performed by a Hewlett Packard 3390A integrator. Calibration plots were prepared by plotting the peak-area ratio (indomethacin/internal standard) against the indomethacin concentration. Calibration standards ranged from 1 to 9 μg/ml indomethacin. Samples were prepared by dissolving 50 mg of each complex in water containing 2×10^{-4} M ammonium hydroxide (used to solubilize). A 0.5 ml aliquot was removed and added to 9.4 ml of methanol to which 0.1 ml of 4.22×10^{-3} M phenylbutazone was added as an internal standard.

The ammonia content of each complex was determined by using a Nessler's reagent calorimetric method (Jenkins et al., 1957). This method has a maximum sensitivity limit of 1 μg/ml ammonia. Samples were prepared by dissolving 50 mg of each complex in 50 ml of water containing 2 ml of Nessler's reagent. Standard solutions were prepared by adding a known quantity of ammonia stock solution (prepared from ammonium chloride) to 2 ml of Nessler's reagent. Solutions were brought to a 50 ml volume with water. The color intensity was estimated visually in triplicate by comparing each sample solution to the standard solutions.

The water content of each complex was determined using thermogravimetric analysis (TGA) with the DUPONT 1B/1091 Thermal Analyzer. Analysis was performed by first computing the total percent weight loss from the thermal transition occurring from 25 to 130 °C and subtracting the percent ammonia content to yield the percent water content. This approach was used because TGA was not specific for water loss and the total weight loss was attributed to water and ammonia. Samples were desiccated over calcium sulfate for 72 h prior to assay to remove surface absorbed water. Samples were prepared by placing a 14–16 mg sample into a platinum boat. The nitrogen flow rate was 100 ± 10 ml/min and the heating rate was 5 °C/min.

2.4. Physicochemical characterization

Each complex was subjected to ultraviolet (UV), infrared (IR), proton nuclear magnetic resonance (NMR), and X-ray diffraction spectral analyses. In addition, differential scanning calorimetry (DSC) was used to characterize the thermal behavior.

The UV absorbance spectrum for each complex was obtained using a Bausch and Lomb Spectronic 2000 spectrophotometer. Samples were prepared by dissolving indomethacin and each complex in 2×10^{-4} M ammonia hydroxide solution to obtain a 2.2×10^{-4} M indomethacin solution. The samples were scanned for absorbance from 400 to 200 nm. The IR spectrum for each complex was obtained using a Perkin-Elmer 1420 ratio-recording spectrometer. Samples were prepared by the potassium bromide disk method and scanned for absorbance from 4000 to 650 cm^{-1} . Nuclear magnetic resonance spectra were obtained with a Varian T-60 spectrometer (60 MHz). Samples were prepared by dissolving either 30 mg of indomethacin, β-cyclodextrin, or each complex with 20 mg of anhydrous sodium carbonate (used to solubilize) into 0.5 ml of D₂O containing 0.75% 3-(trimethylsilyl) propionic-2,2,3,3,4,4 acid, sodium salt, as an internal standard. Analysis parameters were as follows: spinning rate 40 rps, sweep time 250 s, sweep width 500 Hz, radio frequency power level 0.05, and spectrum amplitude 40. X-ray diffraction patterns of indomethacin, β-cyclodextrin, and each complex were obtained with a Siemens diffractometer. Samples were prepared using the powder fraction that passed through a 200 mesh and was retained by a 260 mesh sieve, which represented an arithmetic mean particle size of 6.70×10^{-2} mm. The powder fraction was then pressed gently without imparting texture into an aluminum sample holder having a cavity with the following dimensions: 19.0 mm × 6.5 mm × 1.6 mm. Instrumental parameters were as follows: nickel-filtered Cu Kα radiation was used at 30 kV and 28 mA, time constant (s) 10, intensity (counts/s) 2×10^2 , scanning rate 2°/min, chart speed 1 cm/min, and scan range 6–40° of the diffraction angle 2θ. Differential scanning calorimetry analysis was performed with the DUPONT 1090B/1091 Thermal Analyzer. Samples were prepared by placing 10–14 mg of sample into an aluminum pan which was covered and crimped for analysis. Samples were desiccated over calcium sulfate

for 72 h prior to assay in an effort to remove surface absorbed water. Thermograms were analyzed qualitatively by examining both the peak temperature and the endothermic transition contour. The nitrogen flow rate was 50 ± 10 ml/min and the heating rate was $5^\circ\text{C}/\text{min}$.

2.5. Solubility studies

2.5.1. Phase-solubility analysis

The phase-solubility experiment was performed by the method reported by Higuchi and Connors (1965) and Connors (1987). 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 four hours in a constant temperature water bath at $25 \pm 1^\circ\text{C}$. 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_0 is the solubility of indomethacin in the absence of β -cyclodextrin:

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (1)$$

2.6. pH uncorrected solubility

The pH uncorrected solubility of indomethacin was determined in simulated gastric fluid without enzymes and in distilled water. Equilibrium solubility was not measured in the dissolution medium because sink conditions were assumed since indomethacin is a weak acid ($\text{p}K_a$ 5.6) and would be almost completely ionized and soluble in a pH 7.2 buffer.

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 $37.5 \pm 1^\circ\text{C}$. Samples were collected after one and three hours and assayed quantitatively for indomethacin and qualitatively for its two hydrolytic degradation products by a previously

reported HPLC method (Casella et al., 1998a). Equilibrium solubility in simulated gastric fluid could not be determined due to rapid indomethacin decomposition.

2.6.1. 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 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 mixture with β -cyclodextrin, and an indomethacin mixture with Avicel PH102^R were filled into #0 gelatin capsules. All capsules contained a 40 mg dose of indomethacin or a 40 mg equivalent of complex.

2.6.2. Dissolution analysis

Dissolution analysis was performed as reported earlier (Casella et al., 1998b) according to the USP XXI method for each prepared formulation and Indocin^R. 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. The dissolution medium was a pH 7.2 phosphate buffer.

2.6.3. Drug administration

Each complex containing an equivalent to 40 mg of indomethacin was filled into a #0 hard-gelatin capsule. The complex and Indocin capsules were administered orally in a four-way crossover study to adult, male-albino, New Zealand rabbits with an averaged weight of 4.0 ± 0.03 kg, and their weight remained constant throughout the study. The capsules were administered by using the methods reported by Eng et al. (1987) and Venho and Eriksson (1986). The animals were fasted 24 h prior to dosing but were allowed access to water. Each animal was placed in a body-restraint device, which exposed the animal's head. One operator restrained the body and hind legs of the animal to avoid injury. The second operator gently lifted apart the gums with a wooden-tongue depressor and applied a gentle tension just behind the upper and lower incisors to open the mouth partially. The capsule was administered by the second operator advancing a loaded Pet Piller through the opening created

behind the incisors to the back of the pharynx at the tongue root. The plunger was then pushed to deposit the capsule. The Pet Piller is a rubber-tipped, finger-controlled plunger used for dosing animals. By placing the capsule at the back of the pharynx, the investigators assured the capsule was swallowed intact; thus, preventing the rabbit from bringing the capsule forward in the oral cavity where it could have been either chewed or spat out. Following capsule administration, one milliliter of water was given orally by syringe to facilitate swallowing and to prevent the capsule from sticking to the animal's throat.

Blood samples were drawn by venipuncture at 1, 3, 6, 9, 12, 15, 24, 27, and 50 h. The last sample was used to confirm that indomethacin had been eliminated from the blood to below the analytical-detection limit prior to further use of the same rabbit. Blood samples were taken with the animal placed in a head-and-body restraint cage that allowed easy access to the ears. Xylene was applied to the shaved marginal-ear vein, which caused this blood vessel to dilate. The vein was punctured with a 27-gauge needle and the blood was allowed to drip into an open Vacutainer® tube that contained 0.2 ml of 0.105 M sodium citrate buffered with citric acid. The citrate buffer acted as a coagulant by chelating calcium. Each Vacutainer tube was coated with a non-wettable lining to shield the blood sample from glass-activated clotting.

2.6.4. Preparation of blood samples for assay

Blood samples were centrifuged for 10 min at about $400 \times g$ to obtain plasma. A 0.2 ml plasma aliquot was removed and mixed with 0.9 ml of a 44:56 ratio of methanol:acetonitrile solution to precipitate plasma proteins. Additionally, 0.1 ml of 4.9×10^{-5} M phenylbutazone, dissolved in methanol, was added to each sample as an internal standard. This mixture was kept at 4 °C for an hour before being centrifuged for 10 min at $10,000 \times g$. The supernatant was removed, transferred to a test tube, and evaporated to dryness under nitrogen in a 30 °C water bath. The residue was reconstituted with 0.5 ml of a 65:35 ratio acetonitrile:water solution.

2.6.5. Indomethacin assay in plasma and validation

Reconstituted residues were assayed for indomethacin content by a specific HPLC method as reported earlier (Casella et al., 1998a) with two

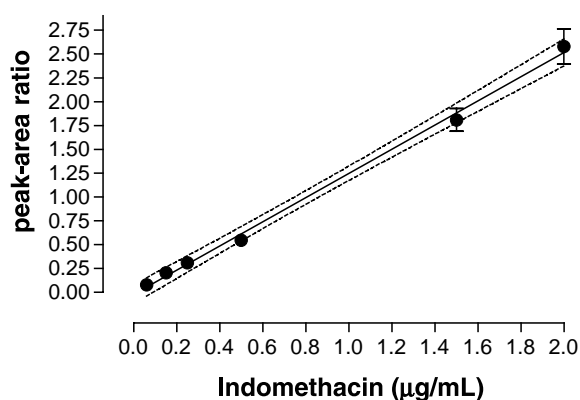


Fig. 1. Calibration curve of indomethacin in plasma.

modifications: (1) a guard column containing the same stationary phase as the HPLC column was added to protect the column from protein adsorption, and (2) the flow rate was adjusted to 1.0 ml/min. Calibration standards ranged from 0.06 to 2 µg/ml indomethacin.

The assay method was validated according to a method reported earlier (Brooks and Weinfeld, 1985). The indomethacin calibration curve ($n = 5$) recovered from rabbit plasma is shown in Fig. 1. The validation data, reported in Table 2, show this method was linear, precise, accurate, and reproducible. The correlation coefficient (r^2) was 0.997, the intercept was -0.021 ± 0.037 , and the slope was 1.3 ± 0.037 . A typical chromatogram of indomethacin and internal standard recovered from rabbit plasma in vitro and in vivo are shown in Fig. 2.

Table 2
Validation of indomethacin assay

Parameter	Value
Linearity (r^2 , $n = 5$)	0.997
Recovery (accuracy)	$85.4 \pm 4.9\%$ ^a
Precision	4.4% ^a
Inter-assay precision (5 weeks) (reproducibility)	Slope: 1.3 ± 0.037 ^b Intercept: -0.021 ± 0.037 ^b r^2 : 0.997
Quantitation range	0.06–2 µg/ml
Detection limit	0.06 µg/ml (60 ng/ml)

^a Relative-standard deviation.

^b Standard error.

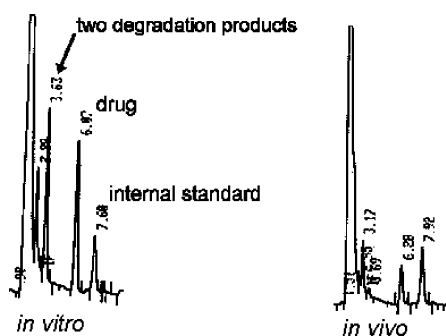


Fig. 2. A typical chromatogram for indomethacin and internal standard.

2.6.6. Software programs

PC Nonlin (Pharsight Corporation, Mountain View, CA) was used to calculate the pharmacokinetic parameters and predicted-plasma profiles. Statgraphics (Manugistics, Inc., Rockville, MD) was used for the ANOVA analyses.

3. Results

3.1. Indomethacin, water and ammonia content

The indomethacin content was about 11% for the β -cyclodextrin complex, and decreased slightly when complexes were formed with the two β -cyclodextrin derivatives as reported in Table 1. Statistical analysis by ANOVA showed the indomethacin content was similar among all three complexes ($P \leq 0.05$).

Assay results show the absence of the degradation products of indomethacin in all the complexes. The complexes were prepared in solutions at about pH 8.0 at 57 °C and, thereafter dried at 70 °C for 12 h. Considering the half-life of indomethacin is about 5 h at 60 °C at pH 8 (Chiba et al., 1992), the authors concluded the cyclodextrins had protected indomethacin from decomposition (hydrolysis). Studies to compare the indomethacin–cyclodextrin complexes to pure indomethacin that had undergone the same process could not be performed because pure indomethacin would have degraded by approximately 80%.

3.2. Ultraviolet spectrophotometric analysis

Ultraviolet spectrophotometric analysis of equal-molar concentrations of the three cyclodextrin

Table 3

Ultraviolet absorbances^a of indomethacin and three cyclodextrin complexes

Sample	Absorbance (318 nm)	Absorbance (265 nm)
β -Cyclodextrin complex	0.202 \pm 0.005	0.523 \pm 0.010
Hydroxyethyl- β -cyclodextrin complex	0.167 \pm 0.006	0.433 \pm 0.019
Hydroxypropyl- β -cyclodextrin complex	0.154 \pm 0.003	0.423 \pm 0.002
Indomethacin	0.147 \pm 0.004	0.377 \pm 0.010

^a Mean \pm S.D. of three determinations.

complexes (2.2×10^{-4} M indomethacin) at lambda maxima of 318 and 265 nm show a linear increase in the absorbance of indomethacin with a change in the substitution pattern of the cyclodextrin as reported in Table 3. A correlation between the ultraviolet absorbance and the cyclodextrin molecular weight ($r^2 = 0.9$ at 318 and 265 nm, respectively) was considered as an evidence of complex formation. No shifting was observed in the two lambda maxima of indomethacin when complexed with the cyclodextrins. The cyclodextrins showed insignificant ultraviolet absorbance.

3.3. Infrared analysis

The infrared spectra of the cyclodextrin complexes were similar to the corresponding pure cyclodextrin and dissimilar to indomethacin and a physical mixture (Casella et al., 1998a) as shown in Figs. 3–9. Minor peaks and peak shifts were observed in the complex spectra when comparisons were made to the corresponding pure cyclodextrin.

The β -cyclodextrin complex showed a broad peak at 1640 cm^{-1} when compared to the corresponding β -cyclodextrin peak at about 1630–1650 cm^{-1} . The broadening of the 1640 cm^{-1} peak was attributed to a change in the hydrated bonds within β -cyclodextrin. The complex spectrum showed three small peaks at 1370, 1330, and 1230 cm^{-1} were attributed to C–H bending, C–O stretching, and O–H deformation absorbencies of indomethacin, and suggested these groups were not included fully within the cyclodextrin cavity.

The hydroxyethyl- β -cyclodextrin complex had a peak at 1670 cm^{-1} that appeared to be shifted from the

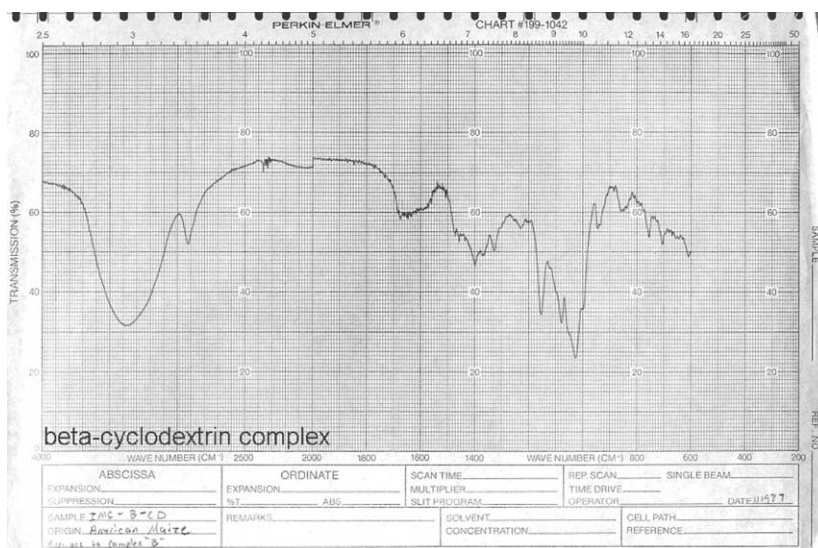


Fig. 3. The infrared spectrum of the β -cyclodextrin complex with indomethacin.

1640 cm^{-1} peak in hydroxyethyl- β -cyclodextrin, and was attributed to a change in the hydrated bonds within hydroxyethyl- β -cyclodextrin. This complex had an absorption peak at 1585 cm^{-1} that was attributed to a C=C stretching absorption of indomethacin, which suggested that this functional group was not fully included within the cyclodextrin cavity.

The hydroxypropyl- β -cyclodextrin complex showed an increase in the relative peak intensity of the 1590 cm^{-1} peak caused by a C=C stretching absorption, when compared to an adjacent, broad peak at about $1640\text{--}1670\text{ cm}^{-1}$. The change in relative intensity was attributed to a diminishing of the 1640 cm^{-1} peak due to a change in the hydrated bonds within

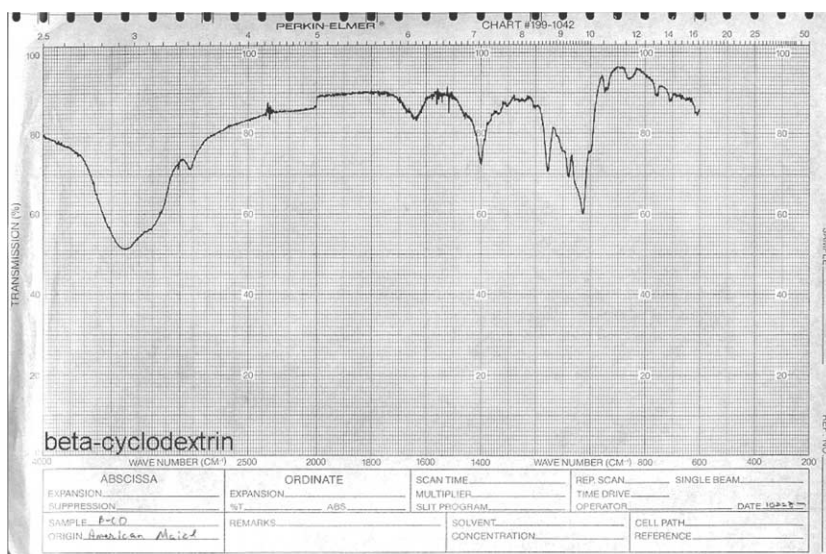


Fig. 4. The infrared spectrum of β -cyclodextrin.

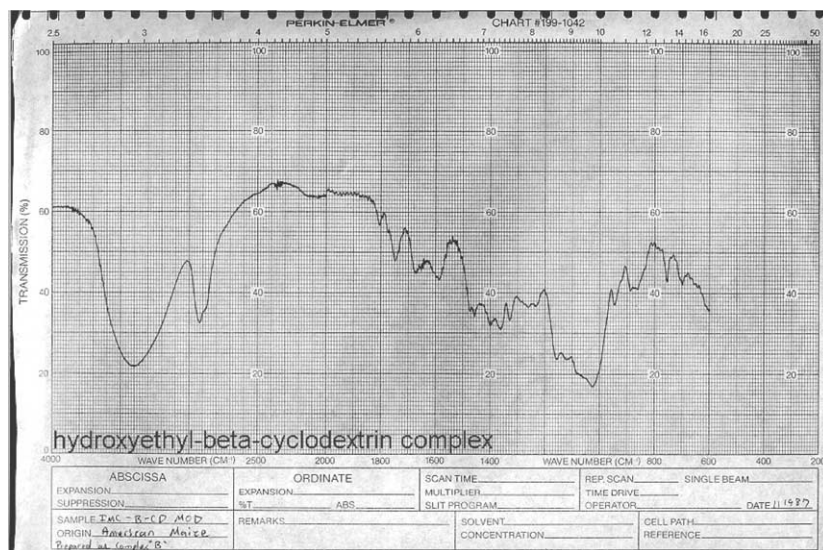


Fig. 5. The infrared spectrum of the hydroxyethyl- β -cyclodextrin complex with indomethacin.

hydroxypropyl- β -cyclodextrin. This complex also had a peak at 1330 cm^{-1} that was attributed to a C–H bending absorption of indomethacin, which suggested this functional group was not included fully within the cyclodextrin cavity.

Considering the infrared spectra of the three cyclodextrin complexes, changes in the water absorbance

at 1640 cm^{-1} were found as evidence of complex formation. The authors postulated the C–H bending and C–O stretching absorbencies suggest the methoxy group and the adjacent aromatic ring of indomethacin were not included fully within the cyclodextrin-ring cavity. The authors attributed the lack of the two intense carbonyl bands of indomethacin in the complex

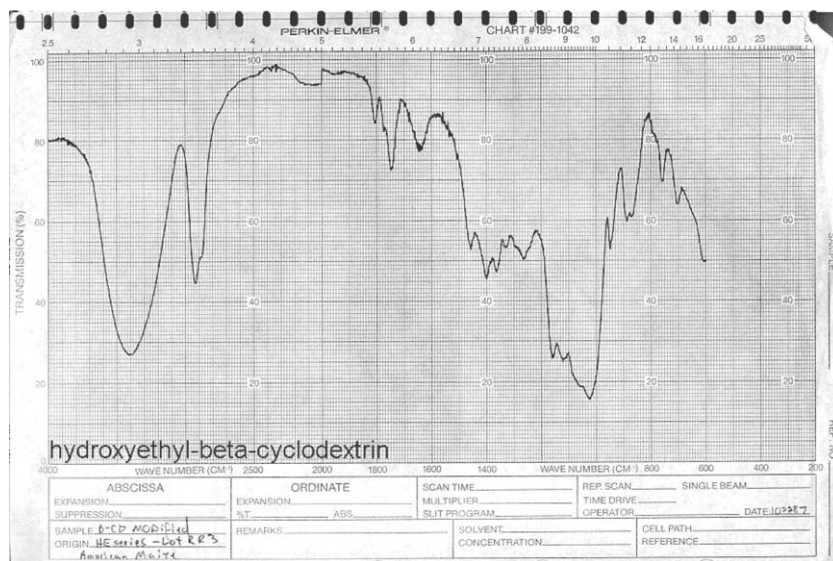


Fig. 6. The infrared spectrum of hydroxyethyl- β -cyclodextrin.

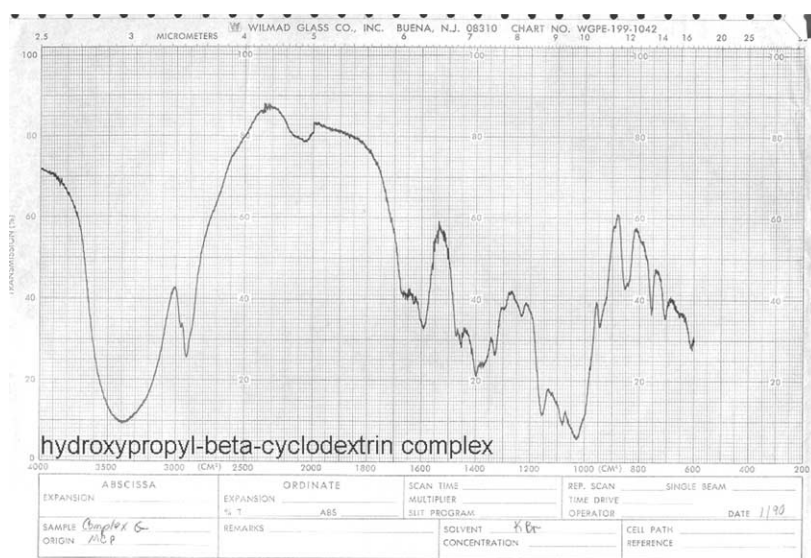


Fig. 7. The infrared spectrum of the hydroxypropyl- β -cyclodextrin complex with indomethacin.

spectra at 1690 and 1720 cm^{-1} to suggest the inclusion of the two carbonyl groups in the cyclodextrin-ring cavity. Previous work using this complex-preparation method had shown the degree of indomethacin inclusion into the β -cyclodextrin cavity could be predicted

by the absence or presence of the two shifted and diminished carbonyl bands (Casella et al., 1998a). The absence of these two bands in all three complexes suggest the two indomethacin carbonyl groups were included and bound well to the cyclodextrins.

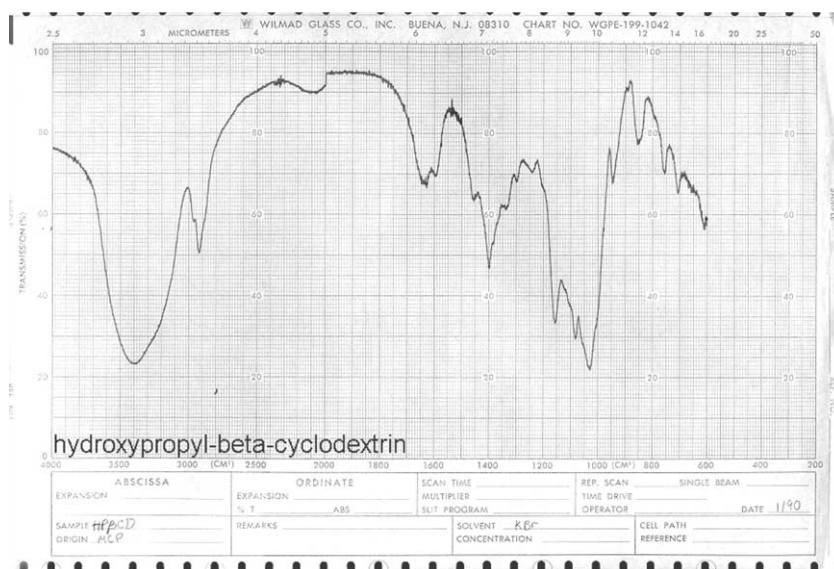


Fig. 8. The infrared spectrum of hydroxypropyl- β -cyclodextrin.

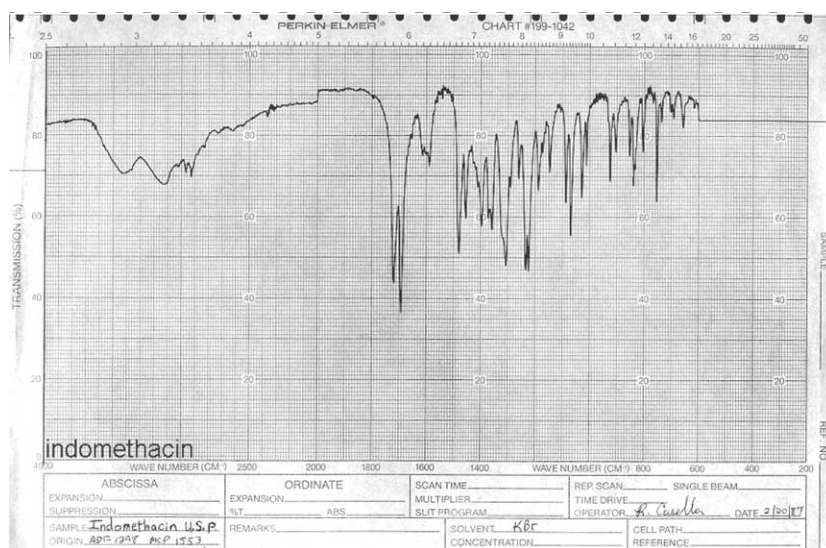


Fig. 9. The infrared spectrum of indomethacin.

3.4. Proton nuclear-magnetic resonance

The chemical shifts of indomethacin, cyclodextrins, and cyclodextrin complexes are reported in Table 4. The appearance of new chemical shifts in the spectra suggests the formation of complex when compared to the indomethacin and pure cyclodextrin spectra. A physical mixture was not evaluated because the authors assumed a complex would form in solution during testing and bias the results.

3.5. Powder X-ray diffraction

Crystalline reflections show indomethacin was crystalline, β -cyclodextrin and its indomethacin

complex were polycrystalline, and hydroxyethyl- β -cyclodextrin, hydroxypropyl- β -cyclodextrin, and the indomethacin complexes with these cyclodextrin derivatives were amorphous as reported in Table 5. The β -cyclodextrin complex was dissimilar to a physical mixture (Casella et al., 1998a) thus suggesting complex formation. Complex formation could not be confirmed using the two cyclodextrin derivatives because their amorphous nature precluded differentiation by crystalline reflections.

3.6. Differential-scanning calorimetry

A comparison among the endothermal transitions of indomethacin, the pure cyclodextrins, and the

Table 4

Proton nuclear-magnetic resonance chemical shifts (ppm) of indomethacin, three cyclodextrins, and three cyclodextrin complexes

Indomethacin	β -Cyclodextrin	Hydroxyethyl- β -cyclodextrin	Hydroxypropyl- β -cyclodextrin	β -Cyclodextrin complex	Hydroxyethyl- β -cyclodextrin complex	Hydroxypropyl- β -cyclodextrin complex
2.10	3.60	3.60	1.00	2.25	2.25	1.00
3.50	3.65	3.70	1.05	3.45	3.60	1.05
4.10	3.90	3.90	3.50	3.65	3.95	3.45
6.15	4.00	4.90	3.70	3.80	5.10	3.70
6.20	4.90	5.10	4.70	4.00	5.30	4.70
6.80	4.95	5.20		4.90		4.80
7.05	5.20			5.00		
7.15				5.25		

Table 5
Crystalline reflections ($2\theta^\circ$) of indomethacin, three cyclodextrins, and three cyclodextrin complexes

Indomethacin	β -Cyclodextrin	Hydroxyethyl- β -cyclodextrin	Hydroxypropyl- β -cyclodextrin	β -Cyclodextrin complex	Hydroxyethyl- β -cyclodextrin complex	Hydroxypropyl- β -cyclodextrin complex
10.10	9.00	Amorphous halo	Amorphous halo	7.00	Amorphous halo	Amorphous halo
11.75	10.50			8.75		
12.75	11.50			10.50		
16.50	12.25			12.25		
17.00	14.50			14.50		
18.50	15.25			15.25		
19.50	16.00			15.75		
21.75	17.00			17.00		
23.25	17.75			17.75		
24.00	18.75			18.50		
25.75	19.50			19.50		
26.50	21.00			20.50		
27.50	21.50			21.00		
28.25	22.50			22.50		
29.00	24.25			24.00		
29.25	25.00			26.75		
30.50	27.00			30.75		
33.00	28.50			35.00		
33.50	31.00					
34.10	32.00					
35.00	34.50					
37.50	35.50					

cyclodextrin complexes show the complex thermograms have shifted-peak temperatures as evidence of complex formation as reported in Table 6. The peak shift occurred in the large, broad endotherm at about 100–120 °C that was attributed to the dehydration of the cyclodextrin-ring cavity as confirmed by thermogravimetric analysis (thermogram not shown). The β -cyclodextrin complex was dissimilar to a physical mixture (Casella et al., 1998a) thus suggesting complex formation.

The β -cyclodextrin complex endotherms at 160 and 154 °C suggest indomethacin polymorphic forms II

and I were present (Borka, 1974). The hydroxyethyl- β -cyclodextrin complex shows no evidence of un-complexed indomethacin. The hydroxypropyl- β -cyclodextrin complex shows evidence of amorphous indomethacin as suggested by a transition at 57 °C (not seen in thermogram because of the scale). The complexes were dissimilar to a physical mixture and had no evidence of a strong indomethacin response at 160 °C from polymorphic form I as reported earlier (Casella et al., 1998a). The authors postulated the small transitions attributed to indomethacin were caused by small quantities of un-complexed indomethacin.

Table 6
Endothermal transitions (°C) of indomethacin, three cyclodextrins, and three cyclodextrin complexes

Indomethacin	β -Cyclodextrin	Hydroxyethyl- β -cyclodextrin	Hydroxypropyl- β -cyclodextrin	β -Cyclodextrin complex	Hydroxyethyl- β -cyclodextrin complex	Hydroxypropyl- β -cyclodextrin complex
160	113	56	110	118	67	57
	222	86	209	154	111	103
				160		236
				220		

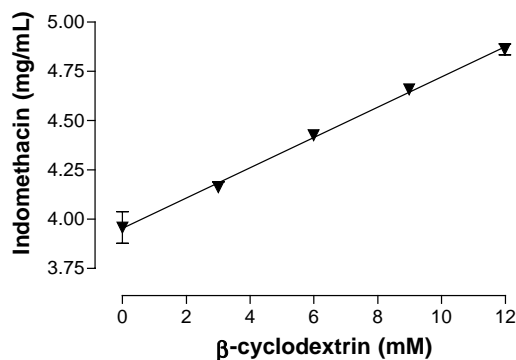


Fig. 10. Phase solubility analysis using β-cyclodextrin.

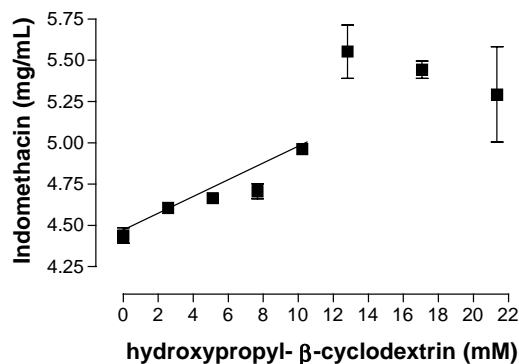


Fig. 12. Phase solubility analysis using hydroxypropyl-β-cyclodextrin.

3.7. Phase-solubility analysis

The β-, hydroxyethyl-β-, and hydroxypropyl-β-cyclodextrins formed type A₁ (1:1 molar ratio) complexes with indomethacin as shown in the phase-solubility plots in Figs. 10–12. The hydroxypropyl-β-cyclodextrin complex had a type-B segment considering the plateau region from 13 to 22 mM as shown in Fig. 12. The binding constants were calculated from the type-A segment, and were found to be similar among the three complexes as shown in Fig. 13.

3.8. pH uncorrected solubility

Indomethacin solubility was increased greatly in distilled water and in 0.1N hydrochloric acid when complexes were formed with either hydroxyethyl- or hydroxypropyl-β-cyclodextrin as reported in Tables 7

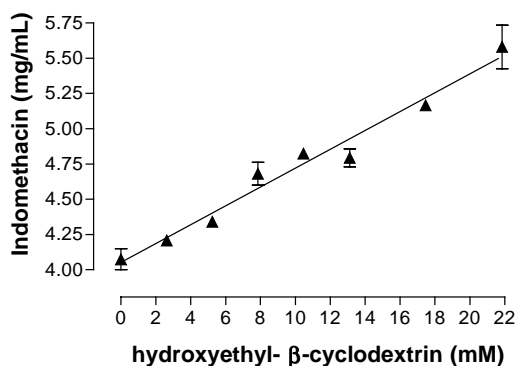


Fig. 11. Phase solubility analysis using hydroxyethyl-β-cyclodextrin.

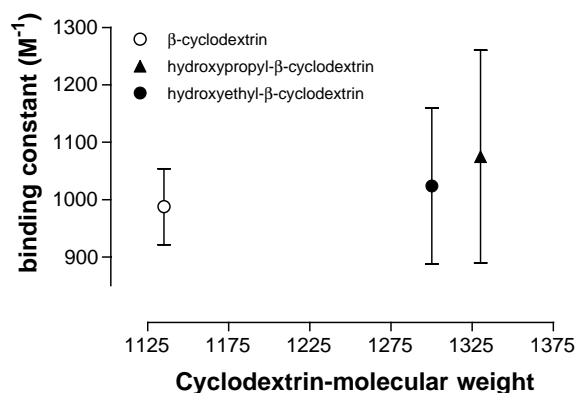


Fig. 13. Binding constants for three β-cyclodextrin complexes.

and 8. Samples were taken at 0.5 and 3 h because indomethacin degraded rapidly in the gastric fluid and no equilibrium could be attained, and equilibrium was attained in water within the error of the measurements. A modest increase in solubility was achieved in both media when a complex was formed with β-cyclodextrin.

Table 7

Indomethacin and cyclodextrin complex solubilities in distilled water (mg/ml)^a

Sample	0.5 h	3.0 h
Indomethacin	0.015 ± 0.001	0.015 ± 0.001
β-Cyclodextrin complex	2.00 ± 0.09	2.10 ± 0.07
Hydroxyethyl-β-cyclodextrin complex	8.04 ± 1.77	8.67 ± 1.98
Hydroxypropyl-β-cyclodextrin complex	9.87 ± 0.52	11.0 ± 1.43

^a Mean ± S.D. of three determinations.

Table 8

Indomethacin and cyclodextrin complex solubilities in 0.1N hydrochloric acid ($\mu\text{g/ml}$)^a

Sample	0.5 h	3.0 h
Indomethacin	1.37 \pm 0.05	2.10 \pm 0.41
β -Cyclodextrin complex	46.1 \pm 5.36	37.3 \pm 3.12
Hydroxyethyl- β -cyclodextrin complex	156.1 \pm 47.37	386.4 \pm 100.8
Hydroxypropyl- β -cyclodextrin complex	648.3 \pm 53.25	278.1 \pm 72.37

^a Mean \pm S.D. of three determinations.

Chromatograms (not shown) from the 0.1N hydrochloric acid samples show the qualitative presence of indomethacin's two degradation products *para*-chlorobenzoic acid and 5-methoxy-2-methylindole-3-acetic acid. The authors postulated the cyclodextrins had hydrolyzed in the presence of acid and caused drug degradation. The pure drug was insoluble in the acid, and no degradation products were observed in solution. Chromatograms from the distilled water samples show no evidence of indomethacin's degradation products.

3.9. Dissolution analysis

The encapsulated hydroxyethyl- β -cyclodextrin complex (hereafter, all encapsulated complexes are indicated as capsules) had superior dissolution when compared to the hydroxypropyl- β -cyclodextrin and β -cyclodextrin capsules as shown in

Fig. 14. Indomethacin from Indocin[®] capsules dissolved slightly slower than the hydroxyethyl- β -cyclodextrin and β -cyclodextrin capsules, but faster than the hydroxypropyl- β -cyclodextrin capsules. Indomethacin from all capsule formulations dissolved better than capsules containing a physical mixture as reported earlier (Casella et al., 1998a; profile shown in Fig. 14).

3.10. Indomethacin pharmacokinetics

The plasma concentration-time profiles for the three cyclodextrin capsule formulations and Indocin capsules are shown in Figs. 15–18. The profile for the β -cyclodextrin and hydroxyethyl- β -cyclodextrin capsules (Figs. 15 and 16) were comparable to Indocin capsules (Fig. 18). The hydroxypropyl- β -cyclodextrin capsules (Fig. 17) were distinct from the Indocin, β -cyclodextrin, and hydroxyethyl- β -cyclodextrin capsules (Figs. 15, 16 and 18). Figs. 15–18 all lacked a smooth profile, which the authors attributed to a prolonged drug adsorption due to the enterohepatic recirculation of indomethacin (Shen and Winter, 1972).

The time of maximum plasma concentration (T_{max}) of Indocin capsules (control) was about 9 h. Other investigators (Kuroda et al., 1983; Nambu et al., 1978; Ohnishi et al., 1986) have reported the time of maximum-plasma concentration (T_{max}) of indomethacin was 0–2 h after administration to a rabbit by intravenous, rectal, or oral-suspension dosage

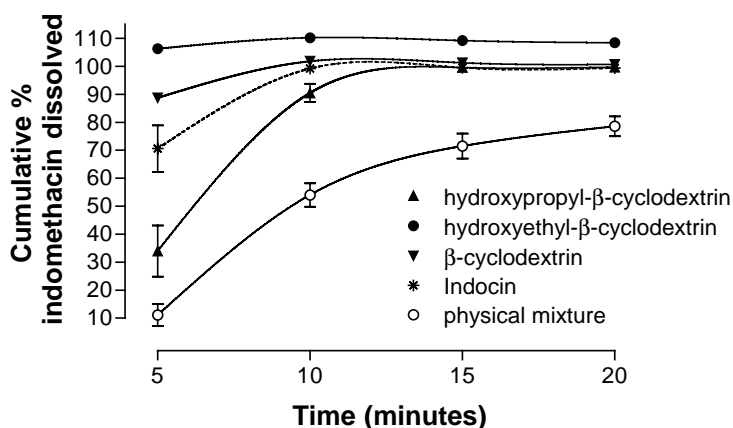


Fig. 14. Capsule dissolution profiles.

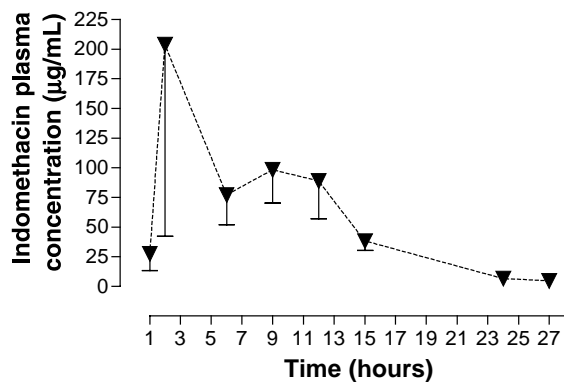


Fig. 15. β-Cyclodextrin capsules.

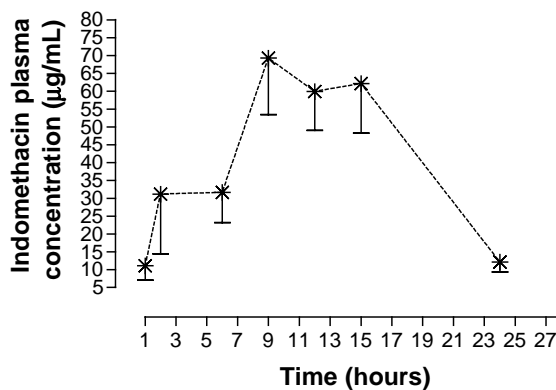


Fig. 18. Indocin capsules.

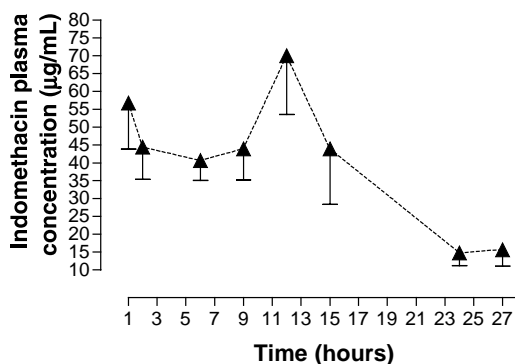


Fig. 16. Hydroxypropyl-β-cyclodextrin capsules.

forms. The peak time (T_{max}) found in this study was believed to be due to slow drug adsorption from the capsule-dosage form because drug absorption was dissolution-rate limited.

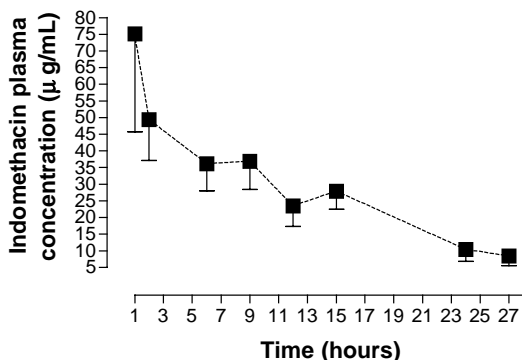


Fig. 17. Hydroxypropyl-β-cyclodextrin capsules.

The absorption-rate constant, elimination rate constant, and the apparent-distribution volume were estimated using a first-order model (Gibaldi and Perrier, 1982) for extra-vascular administration of a drug where absorption and elimination are first-order processes as follows:

$$C_p = \frac{K_a F X_0}{V(K_a - K)} (e^{-Kt} - e^{-K_a t}) \quad (2)$$

where C_p is the plasma concentration at any time (t), K_a is the absorption-rate constant, F is the fraction drug absorbed, X_0 is the administered dose, V is the distribution volume, and K is the elimination-rate constant. The area-under-the-curve from time zero to infinity (AUC_0^∞) is obtained by using Eq. (2):

$$(AUC)_0^\infty = \frac{F X_0}{V K} \quad (3)$$

The half-life was calculated as follows:

$$T = \frac{0.693}{K} \quad (4)$$

The average absorption and elimination-rate constants obtained by using equation 1 yielded a small or no difference between K_a and K for indomethacin from Indocin[®] and cyclodextrin capsules as reported in Table 9. The first-order model assumes K_a is to be much greater than K . Infrequently, however, drugs can have a K_a value that may approach or be equal to the K value; thus, a first-order model becomes imprecise. Under such conditions, Eq. (4) which describes a compartment-independent model for drug pharmacokinetics as a function of time (Gibaldi and Perrier,

Table 9

The pharmacokinetic parameters of indomethacin released from β -cyclodextrin, hydroxyethyl- β -cyclodextrin, hydroxypropyl- β -cyclodextrin, and Indocin capsules as determined by a first-order model

Parameter	β -Cyclodextrin capsules	Hydroxyethyl- β -cyclodextrin capsules	Hydroxypropyl- β -cyclodextrin capsules	Indocin capsules
Dose (mg)	40	40	40	50
K_a (h)	0.19 ± 0.11	4.07 ± 3.85	6.79 ± 4.10	0.10 ± 0.03
K (h)	0.19 ± 0.12	0.05 ± 0.02	0.14 ± 0.13	0.14 ± 0.12
AUC (mg h/l)	2090 ± 1063	1655 ± 1066	921 ± 204	1617 ± 512
T_{max} (h)	6.7 ± 3.0	3.0 ± 3.0	1.7 ± 2.2	10.3 ± 3.9
C_{pmax} (μ g/ml)	171 ± 211	60 ± 25	100 ± 92	61 ± 19
Volume, (V/F)	0.2 ± 0.1	0.6 ± 0.2	0.8 ± 0.7	0.3 ± 0.1
Half-life (h)	4.6 ± 2.2	15.9 ± 5.7	9.4 ± 7.0	6.9 ± 3.1

1982) was used to determine the pharmacokinetic parameters as follows:

$$C_p = \frac{k'FX_0te^{-k't}}{V} \quad (5)$$

where, $k' = K_a = k$.

The compartment-independent $(AUC)_0^\infty$ is obtained by using Eq. (5):

$$(AUC)_0^\infty = \frac{FX_0}{Vk'} \quad (6)$$

Other investigators (Chan and Miller, 1983) proposed that when the difference between K_a and k was very small, a compartment-independent model would be better for describing the drug pharmacokinetics. Another investigator (Patel, 1984) noted that a compartment-independent model could be used to identify the equality of K_a and k , and was a preferred method to calculate the rate constant k' .

Considering the other investigators' conclusions, the plasma-concentration data were fitted to Eq. (4) and the pharmacokinetic data are reported in Table 10. Comparisons among the experimental (Figs. 15–18), predicted first-order model (Table 9), and predicted compartment-independent model (Table 10) plasma-time data show the experimental data agreed best with the compartment-independent model among the four capsule formulations.

We conclude that drug absorption from the Indocin and β -cyclodextrin capsules was slow because drug was either at an absorption site or being eliminated so that little drug was in the body; thus, supporting the finding of equal absorption and elimination rates (Rowland and Tozer, 1989). Alternately, drug absorption from the hydroxyethyl- and hydroxypropyl- β -

cyclodextrin capsules was more rapid since data suggest T_{max} occurred prior to one hour; thus, supporting the finding that this data fit to either a compartment-independent or first-order pharmacokinetic model.

3.11. Bioavailability

The bioavailability was assessed using the pharmacokinetic data determined by the compartment-independent model. The equivalency between the first-order absorption and elimination-rate constants of indomethacin from Indocin and β -cyclodextrin capsules precluded using a first-order model for bioavailability comparisons among the four capsule formulations.

A four-way ANOVA for T_{max} , C_{pmax} , and $(AUC)_0^\infty$ by rabbit shows each test group by capsule formulation was homogeneous ($P \leq 0.10$). The T_{max} and C_{pmax} for indomethacin from capsule formulation were homogeneous ($P \leq 0.10$). The $(AUC)_0^\infty$ for

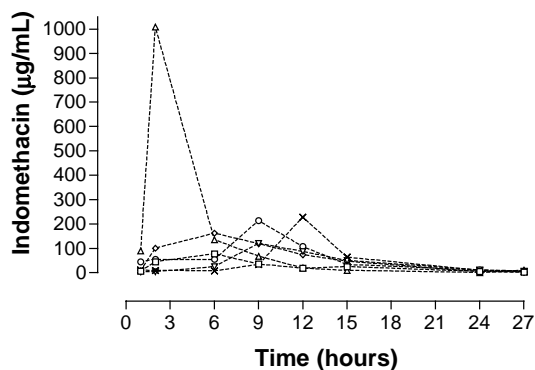


Fig. 19. Plasma profiles for the β -cyclodextrin capsules.

Table 10

The pharmacokinetic parameters of indomethacin released from β -cyclodextrin, hydroxyethyl- β -cyclodextrin, hydroxypropyl- β -cyclodextrin, and Indocin capsules as determined by a compartment-independent model

Parameter	β -Cyclodextrin capsules	Hydroxyethyl- β -cyclodextrin capsules	Hydroxypropyl- β -cyclodextrin capsules	Indocin capsules
Dose (mg)	40	40	40	50
K (h)	0.19 ± 0.12	0.15 ± 0.04	0.31 ± 0.31	0.12 ± 0.06
AUC (mg h/l)	2056 ± 1080^a	1167 ± 618	840 ± 358	1577 ± 506
T_{max} (h)	6.6 ± 3.0	7.0 ± 1.6	6.9 ± 6.8	10.2 ± 3.8
C_{pmax} (μ g/ml)	172 ± 213	60 ± 25	80 ± 69	61 ± 19
Volume (V/F)	0.2 ± 0.1	0.3 ± 0.1	0.8 ± 0.2	0.3 ± 0.1
Half-life (h)	4.6 ± 2.1	4.9 ± 1.1	4.8 ± 4.7	7.8 ± 2.5

^a Statistically significant at $P \leq 0.10$.

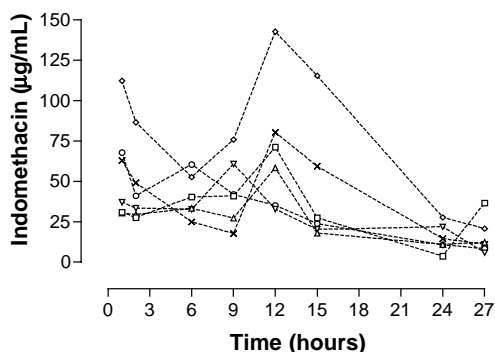


Fig. 20. Plasma profiles for the hydroxyethyl- β -cyclodextrin capsules.

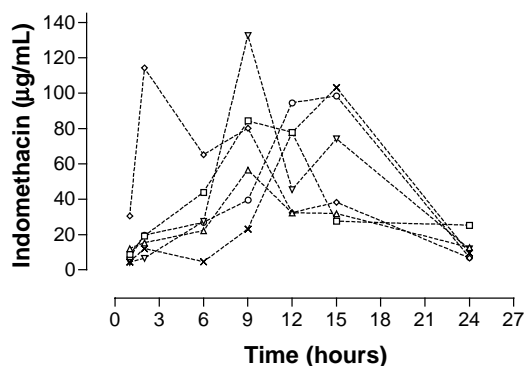


Fig. 22. Plasma profiles for Indocin capsules.

indomethacin from the β -cyclodextrin capsules was found to be significantly higher ($P \leq 0.10$) than the $(AUC)_0^\infty$ for the hydroxyethyl- β -cyclodextrin, hydroxypropyl- β -cyclodextrin, and Indocin[®] capsules. The individual plasma profiles (Figs. 19–22)

for the β -cyclodextrin complex (Fig. 19) show one rabbit had a large $(AUC)_0^\infty$ that was found statistically similar to the other five rabbits in the test group, but biased the result.

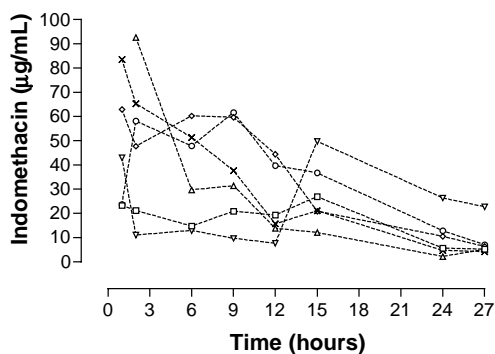


Fig. 21. Plasma profiles for the hydroxypropyl- β -cyclodextrin capsules.

4. Conclusions

Three complexes were prepared successfully with the insoluble drug indomethacin and the water-soluble complexing agents β -, hydroxyethyl- β -, and hydroxypropyl- β -cyclodextrin. An ANOVA showed the indomethacin content among the complexes was statistically non-significant ($P \leq 0.05$). Formation of complexes was confirmed by ultraviolet, infrared, nuclear-magnetic resonance, powder X-ray diffraction, differential-scanning calorimetry, and phase-solubility techniques. Powder X-ray diffraction studies show the β -cyclodextrin complex was polycrystalline in nature; whereas, the hydroxyethyl-

and hydroxypropyl- β -cyclodextrin complexes were amorphous.

The solubility of indomethacin in gastric fluid and water was improved greatly as a result of complex formation with β -, hydroxyethyl- β -, and hydroxypropyl- β -cyclodextrin in comparison to pure indomethacin.

Dissolution studies show the β - and hydroxyethyl- β -cyclodextrin capsules had superior dissolution when compared to hydroxypropyl- β -cyclodextrin and Indocin capsules. Solubility studies show the solubility of complexed indomethacin was much greater than pure indomethacin in water and 0.1N hydrochloric acid. Therefore, the authors expected some improvements in the bioavailability of indomethacin in complexed form. The *in vivo* studies yielded plasma-time profiles that fit best to a compartment-independent model. A first-order model could not be used to describe the pharmacokinetics of β -cyclodextrin and Indocin capsules because slow drug absorption and the enterohepatic re-circulation of indomethacin lead to equal absorption and elimination rates. Capsules containing the β -cyclodextrin complex had a significantly ($P \leq 0.10$) higher $(AUC)_0^\infty$ when compared to all other indomethacin capsule formulations.

No correlations were found among the bioavailability, equilibrium solubility, and dissolution for all complexes. Considering the dissolution profiles, one could have postulated the β - and hydroxyethyl- β -cyclodextrin complexes would have had the highest relative bioavailability. Considering the solubility studies, one could have postulated the hydroxypropyl- and hydroxyethyl- β -cyclodextrin complexes would have had the highest relative bioavailability. Since neither dissolution nor solubility studies were able to predict the relative bioavailability, nor was any correlation found among the binding constant measured by phase solubility analysis, the authors concluded that other factors were responsible for the differences among the formulations.

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