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Inclusion Complex of Carprofen with Hydroxypropyl-β-cyclodextrin

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Abstract

The aim of this study was to investigate the characteristics of hydroxypropyl-β-cyclodextrin (HPCD) on the solubility, photostability and dissolution of carprofen (CP). It was found that the solubility of carprofen increased 52-fold when 16% HPCD was added to H_2O (w/v). The phase-solubility diagram revealed the formation of a 1 : 1 inclusion complex of CP-HPCD with a stability constant (k_s) of 487 M^{-1}. Formation of the inclusion complex of CP-HPCD was analyzed using differential scanning calorimetry (DSC). Changes in chemical shifts of the ^{1}H-nuclear magnetic resonance spectra of CP-HPCD demonstrated that the inclusion site of CP by HPCD was carbazolyl aromatic ring skeleton rather than the side chain of propanoic acid. The photostability study revealed that the CP-HPCD complex could not significantly decrease the rate of photodegradation of CP, implying that the rate-determining step of CP mainly occurred at the side chain. The dissolution rates of CP were significantly enhanced as the proportions of HPCD increased in the prepared discs. The dissolution of the physical mixture (in a 1 : 3 molar ratio) increased by about 6-fold in comparison with the parent drug. The improvement of wettability and solubility of CP by complexing to HPCD was reflected in the enhanced dissolution rate.

Introduction

Carprofen (CP), 2-(6-chloro-2-carbazolyl) propanoic acid (Figure 1), is a non-steroidal anti-inflammatory drug (NSAID) which is used to treat patients with rheumatoid arthritis, osteoarthritis, and acute gouty arthritis [1–3]. However, the poor aqueous solubility of CP in water has led to limitations in developing useful dosage forms. Poor aqueous solubility along with lower dissolution behavior is also believed to contribute to its low bioavailability [4]. When CP is administered orally, the adverse effect of gastric ulceration is involved [5, 6]. Ulceration of the stomach may be caused by the slow dissolution of NSAIDs resulting in mucosal damage because of high local drug concentrations [7]. In addition, CP is very active in producing photosensitized allergic and hemolytic adverse effects [8, 9]. Thus, improving the photostability of CP is synonymous with reducing its phototoxicity.

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide of seven glucose residues with a cavity capable of forming an inclusion complex with several poorly water-soluble compounds. The modified physicochemical properties thereby enhance their solubility, stability, and dissolution rate in an aqueous environment [10, 11]. The drawbacks of low aqueous solubility (about 1.8% w/v at 25 °C), hemolytic activity, and potential for renal toxicity upon parenteral administration [12, 13] have limited the widespread use of β-CD. One means of enhancing the aqueous solubility and reducing the toxicity of β-CD without interfering with its ability to form inclusion complexes is chemical derivation [14]. As a better option, hydroxypropyl-β-cyclodextrin (HPCD), a derivative of β-CD, is a highly water-soluble (>100%, w/v) amorphous cyclodextrin, which retains the ability to form inclusion complexes, but is devoid of any significant toxicity [10, 11].

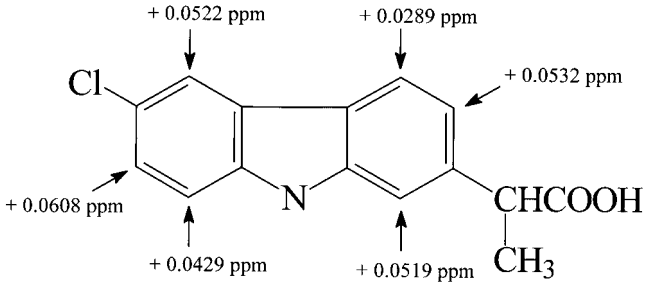

Figure 1. Chemical structure of CP and influence of HPCD on the ^{1}H-NMR chemical shift of CP.

In order to overcome the shortcomings of CP, the aim of this study was to improve the solubility, photostability, and dissolution rate of CP by complexing it to HPCD. The inclusion complex of CP-HPCD was also characterized using DSC and ^{1}H-NMR techniques.

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Experimental

Materials

Carprofen (Sigma Chemicals, St. Louis, MO, USA) and HPCD ($[\alpha]^{25}$ = + 127°, c = 1, H_2O) with Ave. MS 0.8 (Aldrich Chemicals, St. Louis, MO, USA) were used. LC-grade acetonitrile and ethanol were from Fisher Chemicals (Springfield, NJ, USA). The Cosmosil 5C_{18}-AR reverse-phase HPLC column (250 × 4.6 mm i.d.) was the product of Nakalai Tesque (Kyoto, Japan). All other chemicals were of analytical reagent grade.

Phase-solubility studies

The purity of CP was monitored by a stability-indicating HPLC assay method [15], and no appreciable contaminants could be detected. Phase-solubility studies were carried out according to the method reported by Higuchi and Connors [16]. Excess amounts of CP were placed in individual 15-ml screw-capped vials. To each vial, 5 ml of distilled water containing various concentrations of HPCD (0–16%) was added. The vials were sonicated in an ultrasonic bath (Branson 5210, USA) for 30 min and placed in a rotating apparatus (Fargo Instrument, Taiwan) at a rotating rate of 100 rpm. The temperature was maintained at 25.0 ± 0.1 °C for 48 h. An aliquot of the solution was withdrawn and filtered through a 0.45-μm Millipore film. Prior to the quantitation of CP, it was determined that no appreciable absorption of HPCD, HPC, talc, or lactose could be observed at 260 nm. Thus, UV spectrometry at 260 nm was selected to prevent possible interference from the excipients added. The concentration of CP in each solution was determined by UV absorption analysis in triplicate at 260 nm with a Hitachi U-2000 spectrophotometer (Tokyo, Japan).

Preparation of the physical mixture and inclusion complex

The exact amounts of CP and HPCD (in a 1 : 1 molar ratio) were carefully weighed and ground in a ceramic mortar to prepare the physical mixture. The inclusion complex was prepared by the freeze-drying method [7]. The equilibrium solutions obtained from the phase-solubility studies were lyophilized and kept in a desiccator before use.

Characterization of the physical mixture and inclusion complex

The DSC thermograms of CP, HPCD, the physical mixture (in a 1 : 1 molar ratio), and the freeze-dried inclusion complex (CP-HPCD) were recorded on a Setaram TG-DSC 111 thermal analyzer (Caluire, France) equipped with a DSC cell using nitrogen as the purging gas. Each sample was subjected to DSC at a scanning speed of 10 °C/min from ambient temperature to 280 °C. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra of CP and CP-HPCD were registered in CD_3OD on a Brüker DRX-500 NMR spectrometer (Rheinstetten, Germany) with an accuracy of 0.0001 ppm.

Table 1. Formulations of CP discs containing different proportions of HPCD for dissolution studies[a]

Formulation	CP	HPCD	Talc (lubricant)	HPC[b] (binder)	Lactose (diluent)
Intact CARP	1.5	0	1.5	1.0	96.0
1 : 1 molar ratio	1.5	8.5	1.5	1.0	87.5
1 : 2 molar ratio	1.5	16.5	1.5	1.0	79.5
1 : 3 molar ratio	1.5	25.0	1.5	1.0	71.0

[a] All units expressed in percent (%).
[b] HPC: hydroxypropyl cellulose.

Photodegradation of CP and CP-HPCD

A 50-μg/ml solution of CP and CP-HPCD was prepared in a 20% ethanolic aqueous solution for the purposes of studying the photodegradation behavior. Each 6-ml test solution containing 50 μg/ml of CP and CP-HPCD was transferred to a 20-ml clear glass container and exposed to a fluorescent light (NEC-FL20SSEX-D/18HG). The distance from the light source to the samples was 30 cm (1500 lux). An aliquot of the 500-μl solution was removed at each pre-determined checkpoint. The remaining CP in the solution was assayed with a stability-indicating HPLC assay method as previously reported [15].

Dissolution rate studies

In order to study the dissolution rates, constant surface-area discs were made up according to the formulations listed in Table 1. CP of 200 mg was accurately weighed, pulverized, and mixed well in a ceramic mortar and then compressed into discs under 500 kg/cm^2 of pressure. Each disc was placed into a 15-ml screw-capped vial, which contained 10 ml of 0.02 M phosphate buffer (pH 2.5). A 0.50-ml aliquot of the solution was removed at each pre-determined checkpoint, and then 0.50 ml phosphate buffer was added back to the solution. The entire solution was filtered through a 0.45-μm Millipore film. The concentration of CP was further measured by UV absorption at 260 nm in triplicate with a Hitachi 2000 spectrophotometer.

Results and discussion

Phase-solubility studies

The phase-solubility diagram of CP in the HPCD aqueous solution (0–16%) is shown in Figure 2. The solubility of CP was observed to increase by 52-fold with 16% aqueous HPCD (w/v). The solubility of CP increased linearly as a function of HPCD concentration with a regression equation of $y = 154.105x + 51.573$ ($r > 0.995$), and the solubility curve can be classified as an A_L type [16]. The stoichiometry of the inclusion complex was found to be 1 : 1 (guest:host) based on Figure 2. The apparent stability constant for complex formation (k_s) was calculated to be 487 M^{-1} by applying the following equation:

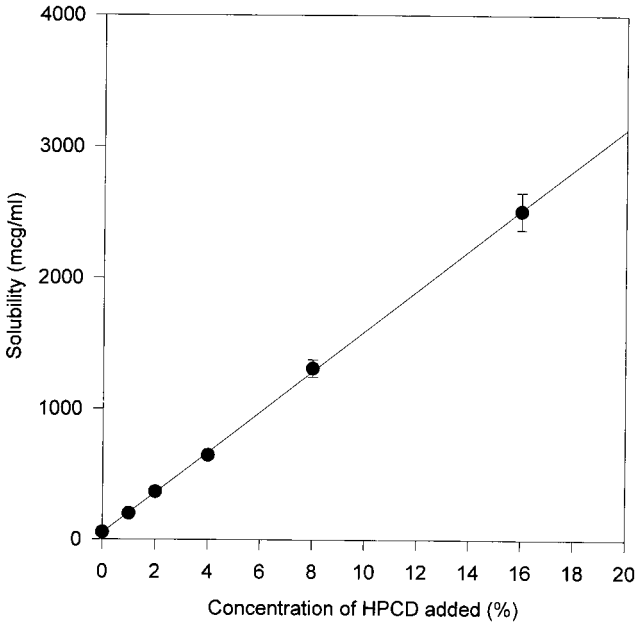

Figure 2. Phase-solubility diagram of CP in an aqueous HPCD solution.

$$k_s = \frac{\text{Slope}}{\text{Intercept} \times (1 - \text{Slope})}.$$

The outer surface of the HPCD molecule is hydrophilic, but the internal cavity is non-polar [17]. The stability constant for complex formation is reflected in the physical acting intensity of CP in the HPCD hydrophobic cavity. A higher complex stability constant for CP-HPCD implies that the hydrophobic nature of CP was responsible for these interactions.

Characterization of the physical mixture and inclusion complex

DSC provided the necessary evidence to verify the formation of an inclusion complex of CP with HPCD. The DSC thermograms of CP, HPCD, the physical mixture (1 : 1 molar ratio), and the freeze-dried inclusion complex are shown in Figure 3a–d. The DSC trace of CP shows one endothermic peak at 202.2 °C, corresponding to its melting point. The exothermic peak of CP at 189.9 °C indicates the beginning of the decomposition process. Two exothermic peaks at 263.1 °C and 273.0 °C could be attributed to the afterward decomposition points of CP (Figure 3a). Meanwhile, the DSC trace of HPCD showed one very broad endothermic peak at 83.1 °C with the loss of water content [19] (Figure 3b). Based on the DSC thermograms, the physical mixture and inclusion complex showed significant differences with respect to CP or HPCD, respectively. In a close examination of the thermogram of the physical mixture, disappearance of the characteristic endothermic and exothermic peaks of CP was found, while new, weak fusion peaks appeared at 141.1, 166.2 and 258.5 °C, indicating that some interactions had occurred between CP and HPCD (Figure 3c). In Figure 3d, the formation of an inclusion complex can be observed, judging from the complete disappearance of all characteristic peaks of CP. Additionally, a shift to a lower temperature of the characteristic endothermic peak of HPCD was found for the inclusion complex (−7.8 °C). The results provide strong indications for the formation of a new solid phase corresponding to the inclusion complex with strong interaction between CP and HPCD.

^{1}H-NMR spectra were further used to characterize CP-HPCD. The chemical shifts of CP were previously reported by Manchand, et al. [18]. In order to determine the conformation of CP in HPCD, changes in magnitudes of the chemical shifts of CP and CP-HPCD were measured. The total complexation of CP with HPCD can be separated into two parts: the non-polar basic skeleton (carbazolyl aromatic ring) and the polar side chain (propanoic acid) of CP. Each part is capable of complexing with HPCD to form a 1 : 1 inclusion complex. As shown in Figure 1, the chemical shifts of protons on the carbazolyl skeleton of CP-HPCD had shifted downfield in a range of from 0.0289 to 0.0608 ppm in comparison with that of CP. The results clearly indicate that the non-polar carbazolyl skeleton of CP had been included in the cavity of HPCD. For the sake of hydrophobic interactions and geometric fittings, a number of reports have demonstrated that the orientation of compounds included into the cavity of β-CD are aromatic skeletons rather than side chains [19, 20].

Photodegradation of CP and CP-HPCD

CP and CP-HPCD were subjected to light exposure to compare their photodegradation behaviors. The photodegradation of CP followed apparent first-order kinetics [15]. The first-order kinetic profile of CP and CP-HPCD are shown in Figure 4. The first-order degradation rates of CP and CP-HPCD were calculated to be 0.113 ± 0.003 and 0.126 ± 0.017 h^{-1}, respectively. Apparently, the photostability of CP was not significantly enhanced through complexing with HPCD. The photodegradation of CP could occur either through the loss of 6-chlorine at the carbazolyl skeleton or by decarboxylation of the side chain propanoic acid [9]. Based on the above results of photodegradation and the ^{1}H-NMR characterization of CP and CP-HPCD, the decarboxylation reaction seems to dominate the photodegradation rate of CP.

Dissolution rate studies

To enhance the dissolution rates of CP, various proportions of HPCD (1 : 1, 1 : 2, and 1 : 3 molar ratios) were added into the disc formulations. The dissolution rate profile of the prepared discs is shown in Figure 5. The results revealed that by increasing the HPCD proportion in the formulation, a faster drug dissolution rate than that of intact CP was observed. In a period of 60 min, the amounts of CP dissolved from the prepared discs containing 1 : 1, 1 : 2, and 1 : 3 molar ratios of HPCD were 15.50%, 26.33%, and 31.42%, respectively, as compared to 5.38% from intact CP. Dissolution of the prepared disc of the physical mixture (in a 1 : 3 molar ratio) increased about 6-fold in comparison with the parent drug alone. The significant enhancement in the CP dissolution

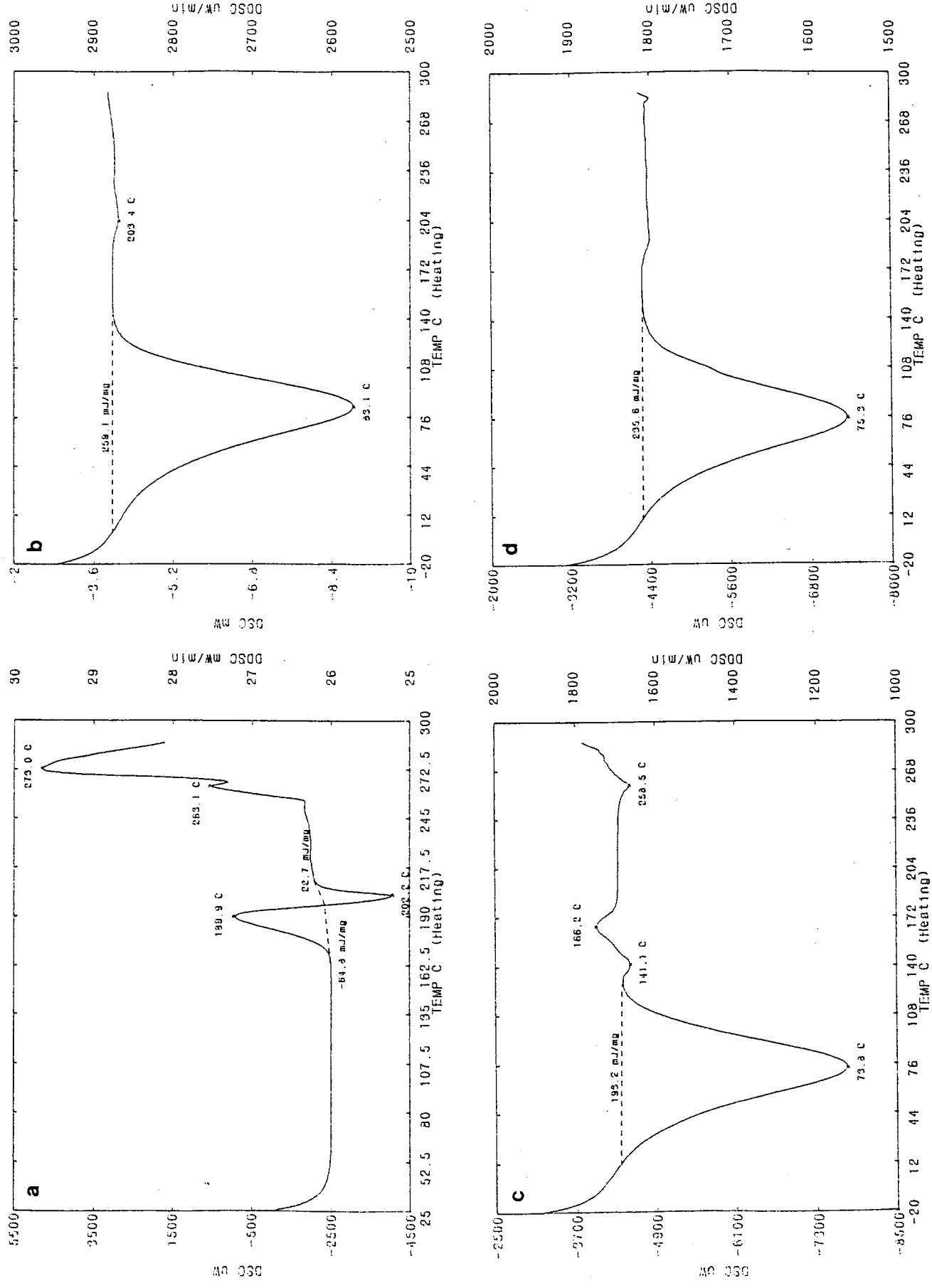

Figure 3. DSC thermograms of CP (a), HPCD (b) a 1 : 1 physical mixture (c), and a freeze-dried inclusion complex (d).

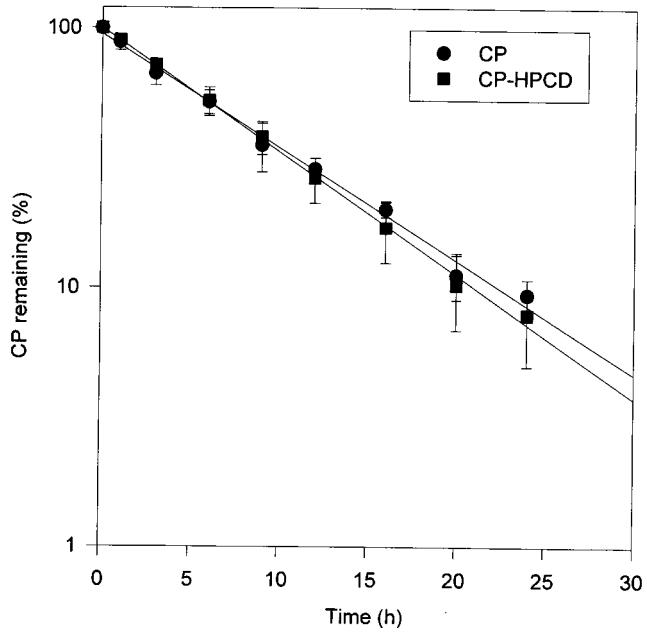

Figure 4. Photodegradation profiles of CP and CP-HPCD.

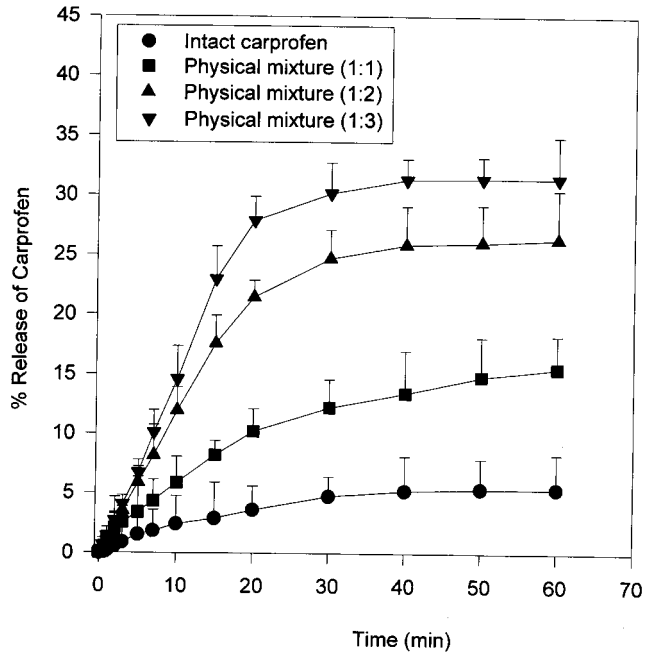

Figure 5. Dissolution profiles of intact CP and physical mixtures (1 : 1, 1 : 2, and 1 : 3) in phosphate buffer solution at pH 2.5.

rate from the prepared disc may be due to local solubilization action in the microenvironment or to hydrodynamic forces attributed to HPCD which improved the wettability [21]. Therefore, enhancement of the dissolution rate was reflected in the improved wettability and solubility by HPCD.

In conclusion, we have demonstrated that HPCD can be used successfully to improve the aqueous solubility and dissolution of CP. Enhancement of the solubility and dissolution of CP was approximately 52-fold at 16% HPCD and about 6-fold at a 1 : 3 molar ratio of CP: HPCD, respectively. The results were attributed to the formation of an inclusion complex as verified by the DSC technique. The non-polar carbazolyl skeleton of CP was oriented in the inclusion cavity of HPCD as determined from the ^{1}H-NMR characterization. Decarboxylation of the side chain containing a propanoic acidic derivative is the rate-determining pathway dominating the photodegradation rate of CP. From a practical point of view, the enhancement of solubility and dissolution may be of great potential in developing suitable dosage forms of CP with higher bioavailability and preventing the adverse effect of gastric irritation.

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References

1. T.F. Yu and J. Perel: *J. Clin. Pharmacol.* **20**, 447 (1980).
2. R.L. Williams, D.E. Furst, H.G. Mandel, D. Nicoll, J.J. Konikoff, and L.Z. Benet: *J. Clin. Pharmacol.* **21**, 493 (1981).
3. W.M. O'Brien and G.F. Bagby: *Pharmacotherapy* **7**, 16 (1987).
4. M.S. Islam and M.M. Narurkar: *Drug Dev. Ind. Pharm.* **17**, 1229 (1991).
5. A. Shiotani, Y. Yamaoka, H.M. El-Zimaity, M.A. Saeed, W.A. Qureshi, and D.Y. Graham: *Dig. Dis. Sci.* **47**, 38 (2002).
6. J.L. Wallace, W. McKnight, B.K. Reuter, and N. Vergnolle: *Gastroenterology* **119**, 706 (2000).
7. N. Erden and N. Celebi: *Int. J. Pharm.* **48**, 83 (1988).
8. L. Becker, B. Eberlein-konig, and B. Przybilla: *Acta Derm. Venereol.* **76**, 337 (1996).
9. G.D. Guidi, R. Chillemi, L.L. Costanzo, S. Giuffrida, and G. Condorelli: *J. Photochem. Photobiol. B: Biols.* **17**, 239 (1993).
10. O. Bekers, E.V. Uijtendaal, J.H. Beijnen, A. Bult, and W.J.M. Underberg: *Drug Dev. Ind. Pharm.* **17**, 1503 (1991).
11. D. Duchêne and D. Wouessidjewe: *Drug Dev. Ind. Pharm.* **16**, 2487 (1990).
12. O. Bekers, J.H. Beijnen, B.J. Vis, A. Suenaga, M. Otagiri, A. Bult, and W.J.M. Underberg: *Int. J. Pharm.* **72**, 123 (1991).
13. S. Choudhury and K.F. Nelson: *Int. J. Pharm.* **85**, 175 (1992).
14. M. Yamamoto, A. Yoshida, F. Hirayama, and K. Uekema: *Int. J. Pharm.* **49**, 163 (1989).
15. A.B. Wu, C.Y. Chen, S.D. Chu, Y.C. Tsai, and F.A. Chen: *J. Chromatogr. Sci.* **39**, 7 (2001).
16. T. Higuchi and K.A. Connors: *Anal. Chem. Instrum.* **4**, 117 (1965).
17. J. Szejtli: *Pharm. Technol.* 36 (1991).
18. P.S. Manchand, D.L. Coffen, P.S. Belica, F. Wong, H.S. Wong, and L. Berger: *Heterocycles* **39**, 833 (1994).
19. K. Harata: *J. Chem. Soc., Chem. Commun*. 546 (1993).
20. K. Uekema, F. Hirayama, T. Imai, M. Otagiri, and K. Harata: *Chem. Pharm. Bull.* **31**, 3363 (1983).
21. M.A. Hassan, M.S. Suleiman, and N.M. Najib: *Int. J. Pharm.* **58**, 19 (1990).