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Interaction of indomethacin with low molecular weight chitosan, and improvements of some pharmaceutical properties of indomethacin by low molecular weight chitosans

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Summary

The interaction of four kinds of low molecular weight chitosans, which were different in molecular weight and degree of deacetylation, were studied in solution and the solid state using an anti-inflammatory drug, indomethacin (IM), as an acidic model molecule. The solubility of IM enhanced with increasing concentration of low molecular weight chitosan, especially C-I which had the lowest molecular weight and least degree of deacetylation. The C-I crystal complex was obtained in a molar ratio of 16 : 1 (IM : C-I) from aqueous solution. The data suggested that the acetyl group and amino group of chitosan played an important role in the complexation. The IM dissolution rates from kneaded mixtures with low molecular weight chitosans were enhanced in the order of C-I $\gg$ C-II > C-III > C-IV (molecular weight: C-IV > C-III > C-II > C-I). The oral absorption rate of IM from C-I kneaded mixture was improved compared with IM alone.

Introduction

Recently, natural polymers such as polysaccharides and proteins have received much attention in the pharmaceutical field owing to their good biocompatibility and biodegradability (Sawayanagi, 1983; Imai, 1989). Among polysaccharides, chitosan, the deacetylated product of alkaline treatment of chitin, is thought to be one of the most useful natural polymers from the viewpoint of effective utilization of natural resources, and has been studied for the carrier material of sustained-release preparations (Miyazaki, 1981; Kawashima, 1985).

We have previously reported that low molecular weight chitosan (LM chitosan, M.W. 3800), a hydrolyzed product of chitosan, makes the surface drug of a powder hydrophilic by dispersion of the drug into chitosan, and thereby enhances the dissolution rate of a poorly water-soluble drug (Shiraishi et al., 1990). Moreover, it is expected that chitosan, a basic polysaccharide, interacts with acidic drugs rather than basic drugs. However, the

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interaction of chitosan with acidic drugs has been scarcely studied because of its low solubility.

The present paper is mainly concerned with the interaction of LM chitosan with indomethacin (IM) as an acidic model drug. In addition, some pharmaceutical properties of the solid dispersion such as dissolution rate and absorption behavior were examined and compared with IM powder.

Materials and Methods

Materials

Indomethacin (IM; γ-form) was donated by Sumitomo Pharmaceutical Co. Ltd (Osaka, Japan). Low molecular weight chitosans (LM chitosans) were supplied by Kurita Water Industries Ltd (Kanagawa, Japan). The physicochemical properties of LM chitosans used in the studies are listed in Table 1. All other reagents and solvents were of analytical grade, and deionized double-distilled water was used throughout study.

Solubility studies

Solubility measurements were carried out according to the method of Higuchi and Connors (1965). Excess amounts of IM were added to an aqueous suspension containing various amounts of LM chitosans, and the suspensions were shaken at 25 ° C. After equilibrium was reached (7 days), an aliquot was centrifuged and filtered through a membrane filter (pore size 0.45 μm, Toyo Scientific Co. Ltd., Tokyo, Japan). A 0.5 ml aliquot of the sample solution was extracted with 10 ml of ethyl acetate from acidified medium, and the organic phase was analyzed spectrophotometrically at 320 nm.

Preparation of solid complex

The solid complex was prepared by mixing appropriate amounts of IM and LM chitosan (C-I). The amounts were calculated from the descending curvature of phase solubility diagrams. For example, IM (30 mg) was dissolved in a 5% aqueous solution (30 ml) of C-I and the solution was shaken at 25 ° C for 7 days. The complex, which precipitated as a microcrystalline powder, was removed by filtration using filter paper with suction and dried under reduced pressure at 30 ° C for 48 h.

Preparation of kneaded mixtures

Kneaded mixtures of IM with LM chitosans were prepared by the following kneading method. IM and LM chitosans in a weight ratio of 1 : 1, 1 : 2 or 1 : 3 were placed in a mortar, then the mixtures were kneaded with 1.2-times their amount of water for 1 h. For example, in the case of an IM-LM chitosan (1 : 2) kneaded mixture, 1 g of IM and 2 g of LM chitosan were kneaded with 3.6 ml of water for 1 h. Drying was carried out in vacuo at room temperature for 48 h. The fraction that passed through a 100 mesh sieve was used in the following experiments. The physical mixture of IM with LM chitosan was prepared by mixing of the powder (< 100 mesh) in a mortar.

Powder X-ray diffraction studies

The powder X-ray diffractometer (Geiger-Flex

TABLE 1

Physicochemical properties of LM chitosans

	C-I	C-II	C-III	C-IV
Molecular weight	3800	7600	14000	25000
Decomposition temperature (° C)	180	250	250	250
Intrinsic viscosity (dl/g) [a]	0.14	0.32	0.65	1.33
Solubility (g/100 ml at 25 ° C)	> 50	< 0.01	< 0.01	< 0.01
pH (at 25 ° C) [b]	6.66	6.24	5.95	6.85
Cationic density (meq./g)	2.8	5.4	5.4	5.4
Degree of deacetylation (%)	66	87	87	89

[a] Measured in 0.2 M acetic acid/0.1 M sodium acetate in 30 ° C.

[b] Measured in 0.01% C-I solution, saturated solutions of other chitosans.

RAD-1A; Rigaku Denki Co. Ltd., Tokyo, Japan) was operated under the following conditions: X-ray, Ni-filtered Cu-K_α radiation; voltage, 40 kV; current, 30 mA; time constant, 1 s; scanning speed, 1°/min.

Differential scanning calorimetry

The differential scanning calorimeter (Thermo Flex Mode DSC10A; Rigaku Denki Co. Ltd., Tokyo, Japan) was operated at a scanning rate of 10°C/min over the temperature range of 50–200°C.

IR spectrometry

IR spectra (model DS-710; Jasco Ltd., Tokyo, Japan) were recorded using potassium bromide pellets and chloroform solution (IM concentration, 0.14 M; triethylamine concentration, 0.014–0.14 M).

^{13}C CP-MAS NMR spectrometry

The high-resolution solid-state ^{13}C NMR spectra at 75.46 MHz were recorded on a Bruker CXP-300 spectrometer by means of the cross polarization-magic angle spinning (CP-MAS) method. The sample was placed in an Andrew-Beams-type unit machined from perdeuterated poly(methyl methacrylate), and spectra were recorded using a Bruker z-32DR ^{13}C-MAS probe. The contact time for cross polarization was 1 ms. The 90° pulse width was 5 μs and repetition time was 4 s. ^{13}C chemical shifts were calibrated indirectly through the use of external benzene (128.5 ppm from tetramethylsilane).

Dissolution studies

The dissolution behaviors of IM, its LM chitosan complex and kneaded mixtures with LM chitosans were examined according to the paddle method (JP XI). The expeerimental conditions were as follows: sample, IM (7 mg, $<$ 100 mesh) or equivalent amount of its complex and kneaded mixtures ($<$ 100 mesh), dissolution medium, 500 ml of water, temperature, 37°C, stirring speed, 100 rpm. The percent of IM dissolved in the medium was determined spectrophotometrically at 265 nm.

In vivo absorption studies

The experimental animals were four male beagle dogs, weighing 11–12.5 kg, fasted for 24 h prior to drug administration. IM or its kneaded mixture with LM chitosan was wrapped in oblate (2 mg/kg as equivalent of IM) and then administered orally together with 20 ml water. At appropriate intervals, 3 ml of blood was withdrawn into heparinized syringes from the cephalic vein of beagle dogs and immediately centrifuged at 3000 rpm to obtain plasma samples.

The concentration of IM in plasma samples was determined using a high-performance liquid chromatograph (HPLC). IM was extracted with 6 ml of ethyl acetate after adding 0.5 ml of phosphate buffer (pH 3) and 1 ml of water to 1 ml of the plasma. The solution (0.5 ml) of mefenamic acid (concentration 10 μg/ml) was used as an internal standard. The evaporated residue of organic phase (5 ml) was redissolved in 0.2 ml of mobile phase, 10 μl of which was subjected to HPLC for determination of IM. The HPLC conditions were as follows: pump and detector, Hitachi L-6000 type equipped with 655A-21 UV monitor (Tokyo, Japan); column, LiChrospher 100, RP-18 (5 μm, 4 mm i.d. × 250 mm, Merck); mobile phase, methanol : 1% acetic acid (3 : 1); flow rate, 1 ml/min; detection, 265 nm.

Results and Discussion

Solubility studies

Fig. 1 shows the equilibrium phase solubility diagrams obtained for IM with LM chitosans in water at 25°C. The solubility of IM in the presence of C-II, C-III and C-IV increased with increasing amounts of LM chitosan and the solubility curve can be classified as type A (Higuchi and Connors, 1965). The solubility of IM increased in the order of C-II > C-III > C-IV. Since chitosan is a basic polysaccharide, the increasing solubility of IM, which is an acidic drug, might have occurred due to the buffer capacity of chitosan. However, C-II, C-III and C-IV have almost the same degree of deacetylation and pH value in solution (shown in Table 1). The only difference among C-II, C-III and C-IV is in the molecular weight. Therefore, the increasing solubility of IM may be due to the

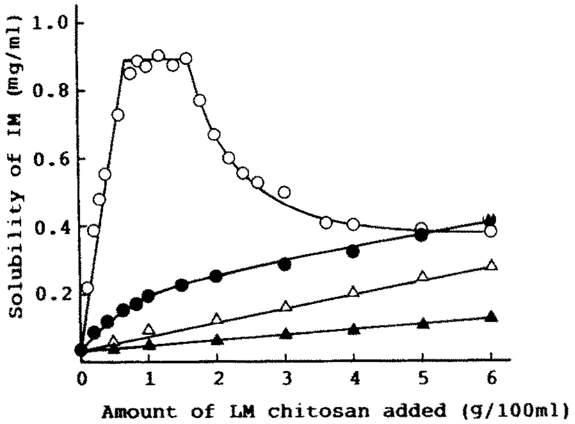

Fig. 1. Phase solubility diagrams of IM-LM chitosan systems in water at 25 °C. (○) C-I system; (●) C-II system; (△) C-III system; (▲) C-IV system. Each point represents the average of 3 experiments.

difference in the interaction mode of IM with LM chitosan, depending upon the molecular weight of LM chitosan. On the other hand, the solubility plot for C-I showed a Bs-type solubility curve. The initial rising portion was followed by a plateau region and finally decreased in total concentration of IM with precipitation of a microcrystalline complex at high C-I concentration. The solubility of IM in the plateau region increased 40-fold as compared to that without C-I. From the result of the chemical analysis of crystalline complexes for IM content, it was found that 16 molecules of IM formed a complex with 1 molecule of C-I (i.e. 1 molecule of IM to 1.5 glucosamine residues). The stability constant (K_c) calculated from initial linear portion according to the Langmuir equation was 14160 M^{-1} based on a 16:1 molar ratio. It was clear that the interaction of IM with C-I was different from those of the other LM chitosans. The molecular weight of C-I is about half that of C-II, while the degree of deacetylation of C-I is remarkably low (66%) compared with other LM chitosans. This result indicates that the acetyl groups in chitosan play an important role in the interaction with IM. Furthermore, the microcrystalline complex appeared yellow, suggesting that the carboxylic acid portion of IM dissociated into ions through the association of the carboxyl group with the amino groups in chitosan.

In a further study of the interaction of IM with LM chitosans in solution, the effects of LM chitosans on the UV spectra of IM were examined and no significant interaction was noted. However, very recent studies at this laboratory showed that the fluorescence of flurbiprofen, an anti-inflammatory drug, was slightly quenched by the addition of glucosamine but not by *N*-acetylglucosamine. This implies that the carboxyl group in flurbiprofen and the amino group in LM chitosan are important in the binding process. Therefore, it is possible to infer from the limited data that LM chitosans interact with IM through hydrophobic and electrostatic interactions. Further investigations on interaction in aqueous solution will be conducted by ^{13}C-NMR and FT-IR spectroscopy, and the results will be reported elsewhere.

Interaction in the solid state

To examine the interaction of IM with C-I in the solid state and to compare it with that of the corresponding physical mixture, X-ray diffractometry, differential scanning calorimetry (DSC), IR spectroscopy and solid state ^{13}C-CP-MAS NMR were employed. Fig. 2 shows the powder X-ray diffraction patterns of the IM-C-I complex, compared with those of the physical mixture, IM and C-I itself. The diffraction pattern of C-I showed a halo pattern over the 2θ range 5–30°. The diffraction pattern of the physical mixture exhibited the characteristic peaks of IM at 17, 19.5, 20.8 and 29.3° at 2θ. On the other hand, the diffraction peaks of the complex were apparently different from IM, and new peaks appeared at around 6, 9, 12, 15 and 16° at 2θ. The diffraction pattern of the complex completely differed from those of IM polymorphs (Otsuka and Kaneniwa, 1988) and chitosan crystals (Saitô, 1987). These data suggested that the crystals of IM disappeared through the complexation and that a new complex crystal was formed.

Fig. 3 shows the DSC thermograms of the IM-C-I complex, the physical mixture, IM and C-I itself. C-I exhibited no endothermic peak owing to its amorphous form. In the case of IM and its physical mixture, an endothermic peak due to the melting of IM was observed around 160 °C. In sharp contrast, the thermogram of the complex

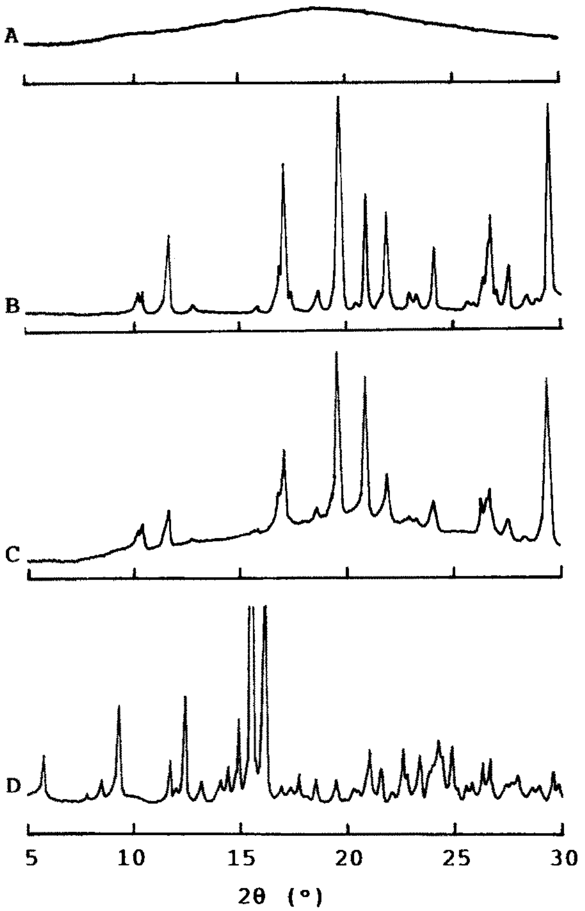

Fig. 2. Powder X-ray diffraction patterns of IM-C-I systems. (A) C-I alone; (B) IM alone; (C) physical mixture of IM and C-I; (D) IM-C-I complex.

showed broad endothermic peaks around 113 and 172°C, which differed from those of IM polymorphs. Although the endothermic peak around 113°C seems to be attributable to the elimination of water, it was observed that water was absent from the complex after drying. Therefore, the endothermic peak around 113°C may be related to the melting of crystallized chitosan through complexation with IM, since the melting points of glucosamine in the α- and β-forms are 88 and 120°C, respectively. Furthermore, the peak around 172°C may be the result of a shift of the melting peak of IM owing to the formation of an ion pair between the carboxylic acid group of IM and the amino group of chitosan. All these peaks indicated the formation of new crystals by the complexation of IM with C-I.

Fig. 4 shows the IR spectra of IM-C-I systems measured by the KBr disk method. The spectrum of the complex showed sharp peaks compared with the physical mixture, indicating the formation of fine crystals. Two sharp peaks were observed at 1715 and 1690 cm^{-1} assigned to the carboxy carbonyl stretching and benzoyl carbonyl stretching bands in IM powder and physical mixture, respectively (Fig. 4B,C). On the other hand, only one peak was observed around 1690 cm^{-1} in the complex (Fig. 4D). The carboxy carbonyl stretching band which tends to shift depending upon the crystal form might shift to low wave number. For example, this band appears at 1735 and 1675 cm^{-1} for α- and β-polymorphic forms,

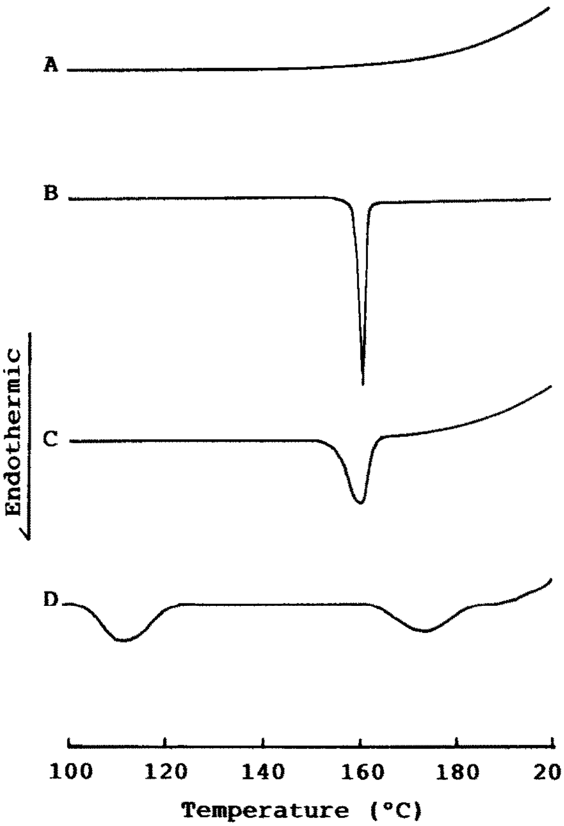

Fig. 3. DSC thermograms of IM-C-I systems. (A) C-I alone; (B) IM alone; (C) physical mixture of IM and C-I; (D) IM-C-I complex.

respectively (Kaneniwa et al., 1985). Furthermore, in the IM crystal, the molecules are strongly linked to their neighbors by hydrogen bonding between carboxyl groups (Kistenmacher and Marsh, 1972). The carboxyl groups in IM crystal were dissociated and might be associated with amino groups through the complexation with C-I. To obtain information about the shift of the carboxy carbonyl stretching band followed by the association with the amino group, IR spectra of IM in chloroform solution were taken in the absence and presence of triethylamine. The carboxy carbonyl stretching band shifted to low wave number with increasing triethylamine concentration, finally overlapping with the benzoyl carbonyl stretching band, as shown in Fig. 4. Thus, the shift of the carboxy carbonyl stretching band in the complex may be explained by the association with the amino group in LM chitosan, although the absorption of the amino group unfortunately could not be observed due to the overlap with the broad absorption peak of the hydroxy group. Moreover, the peaks at 1650 and 1550 cm^{-1} derived from chitosan were remarkably sharp compared with the physical mixture and C-I powder. Since the peak at 1650 cm^{-1} seems to be due to the acetyl carbonyl stretching band of *N*-acetylglucosamine (1640 cm^{-1}), the environment of the *N*-acetyl group of chitosan may be similar to that of the *N*-acetylglucosamine crystal. These data suggested that LM chitosan and IM formed fine crystals through complexation, and that the binding between amino group and carboxyl group played an important role in the complexation.

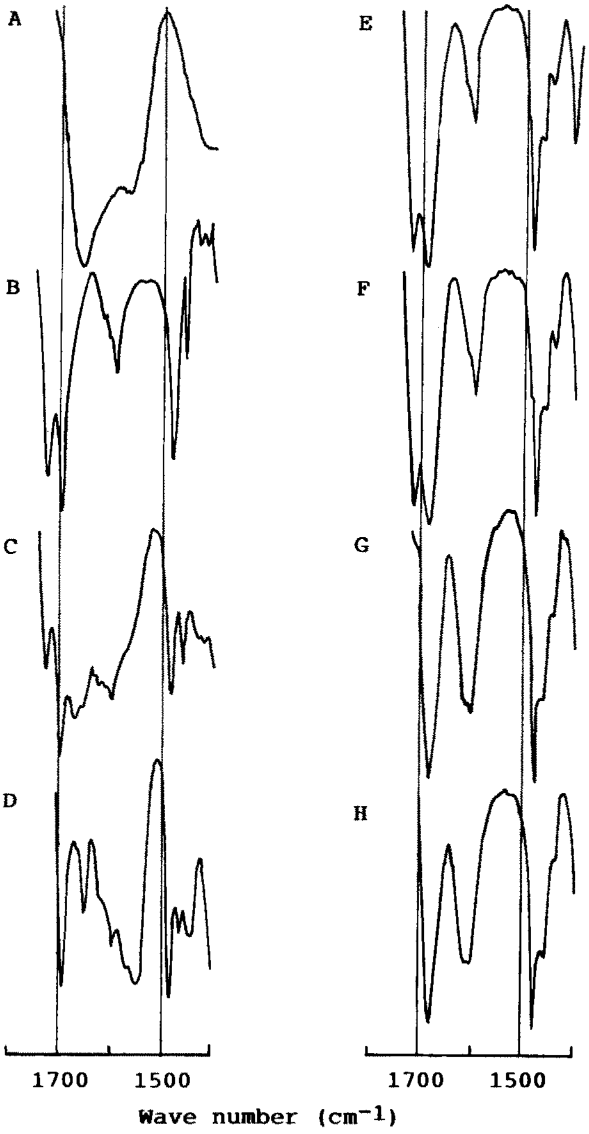

Fig. 4. IR spectra of IM-C-I (left) and IM-triethylamine (TEA; right) systems. (A) C-I alone; (B) IM alone; (C) physical mixture of IM and C-I; (D) IM-C-I complex; (E) IM alone; (F) IM:TEA (1:0.1 molar ratio); (G) IM:TEA (1:0.5 molar ratio); H, IM:TEA (1:1 molar ratio). IR spectra of IM-C-I system were measured by the KBr disk method and those of IM-TEA system were recorded in chloroform solution.

Fig. 5 illustrates the ^{13}C-CP-MAS NMR spectra of C-I-IM systems. The assignments of IM resonances were based on those in solution (Singh et al., 1978), and those of chitosan were according to Saitô et al. (1987). In the case of the complex, no appreciable signals from chitosan molecule were observed under these experimental conditions. Since the ratio of IM and chitosan in the complex was 1:1.5 as a glucosamine unit, C-I peaks should be observed of similar intensity to those of IM. The line-broadening mechanisms may be due to chemical shift dispersion and molecular motion such as exchange among magnetically nonequivalent sites (Lunazzi, 1975; Gierasch et al., 1982), ^{1}H-^{13}C dipolar coupling (Rothwell and Waugh, 1981; Van der Hart et al., 1981) and the resonance frequency via chemical-shift anisotropy (Suwelack et al., 1980; Van der Hart et al., 1981). When chitosan formed a complex with IM, each glucosamine unit in the C-I chain might have been fixed more magnetically nonequivalent. The phase coherence in the transverse ^{13}C magnetisation might be lost as a result of random motional modulation of the resonance frequency via the anisotropic ^{13}C chemical shift. However, details of the disappearance of the chitosan peaks could not be explained clearly. For the signal of IM, the ^{13}C signals of the indole and phenyl ring of IM showed no appreciable change through the binding to chitosan. On the other hand, the other carbons, which do not reside in the ring, were significantly affected by the complexation with chitosan. The acetic acid portion (C-10,11) showed a significant downfield shift, which suggested formation of an ion pair with the amino group of glucosamine. Furthermore, C-10, 12 and 13 showed splitting, indicating that the IM molecule can occupy two or more magnetically nonequivalent positions in the complex. It seems that the free rotation of methyl groups and the acetic acid portion decreased in solution through the complexation with chitosan, and fixed magnetically nonequivalent positions upon crystallization. From the above observations, it was concluded that IM forms a complex with chitosan in water and in the solid state. Moreover,

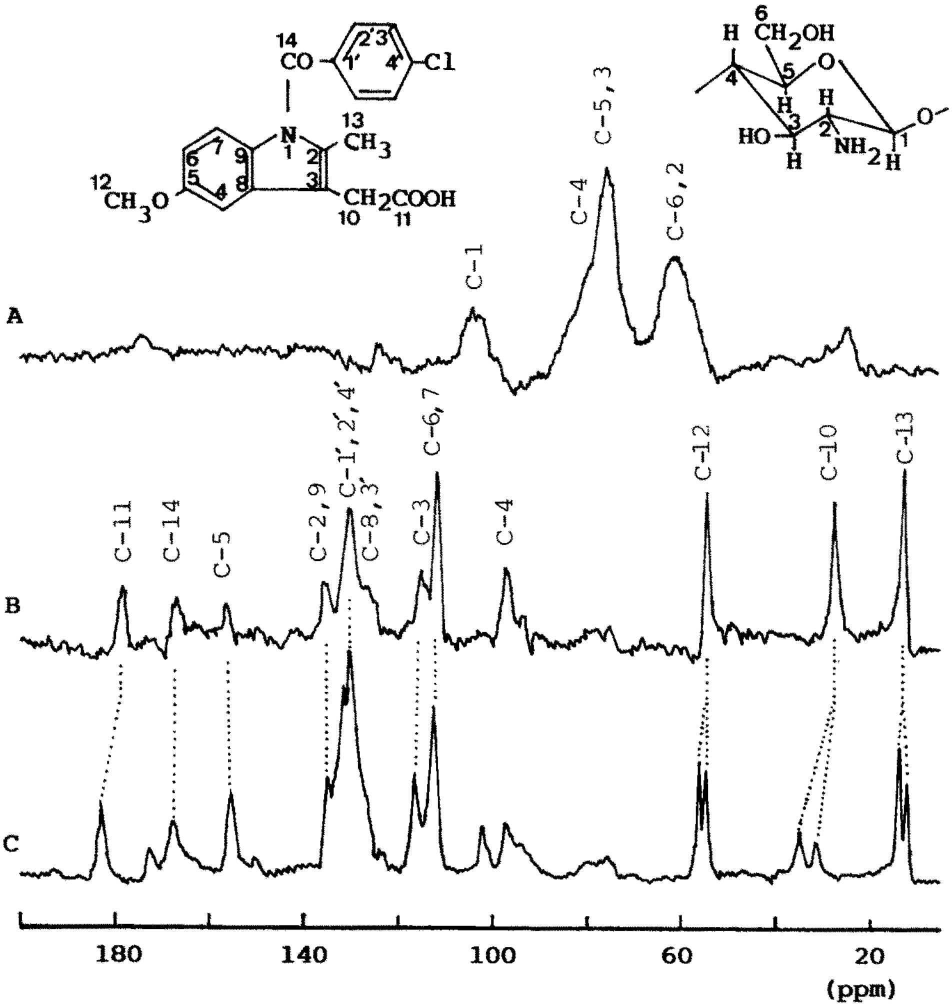

Fig. 5. ^{13}C CP-MAS NMR spectra of IM-C-I complex. (A) C-I alone; (B) IM alone; (C) IM-C-I complex.

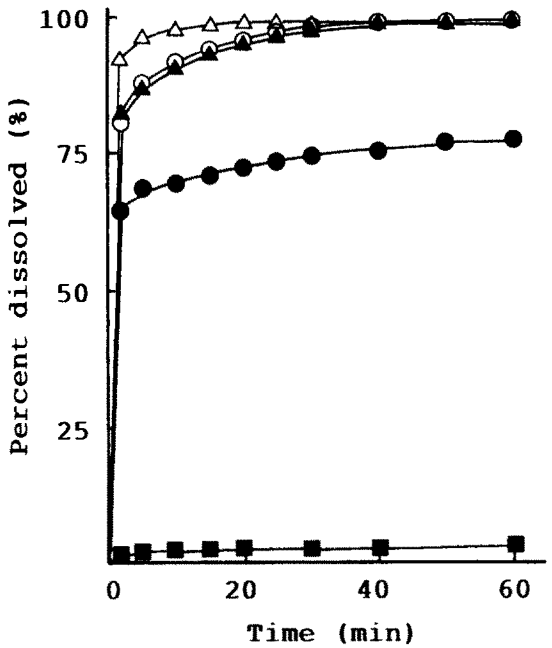

Fig. 6. Dissolution profiles of IM, its kneaded mixtures and complex with C-I in water at 37 ° C. (■) IM alone; (△) IM-C-I complex; (●) IM-C-I kneaded mixture (1 : 1 w/w); (○) IM-C-I kneaded mixture (1 : 2 w/w); (▲) IM-C-I kneaded mixture (1 : 3 w/w). Each point represents the average of 3 experiments.

it was suggested that amino group and acetyl group of chitosan play an important role in the complexation with IM.

Dissolution behavior

Fig. 6 shows the dissolution profiles of IM from its kneaded mixtures containing C-I at three different ratios and the IM-C-I complex in water at 37 ° C. The kneaded mixtures and the complex exhibited a significantly increased dissolution rate compared to that of IM alone. The dissolution rate of complex was the fastest, in spite of the lower content of C-I as compared to those of the 1 : 1 kneaded mixture. It is expected from these results that the dissolution of the 3 : 2 (IM : C-I) kneaded mixture should be inferior to that of the complex. The difference in dissolution rate between the complex and the kneaded mixture may be explained by the difference in the crystal form of IM. In fact, DSC thermograms, IR and X-ray diffraction patterns of the kneaded mixture were identical to those of physical mixture (not shown). These data indicated that IM was dispersed as individual crystals in the kneaded mixture, because the IM particle could not dissolve and disperse monomolecularly in C-I during the kneading process. The rapid dissolution of kneaded mixtures may be due to the improvement in wettability of the powder surface. We previously reported that the dissolution rate of kneaded mixtures was faster than that of physical mixtures with decreasing contact angle on the drug particle (Shiraishi et al., 1990). The dissolution rates of IM from kneaded mixtures were enhanced with increasing amounts of C-I, the 1 : 2 and 1 : 3 (IM to C-I) kneaded mixtures showing almost the same dissolution behavior as the complex. Thus, the pharmaceutical characteristics of the solid complex of IM are more acceptable as compared with those of IM. However, the application of the complex in the pharmaceutical field is rather difficult because of the significantly low yield of the complex. Hence, the 1 : 2 kneaded mixture which showed a rapid dissolution rate and contained smaller amounts of chitosan was more extensively investigated.

Fig. 7 shows the dissolution profiles of IM and its kneaded mixtures with four kinds of LM chitosan (1 : 2 w/w) in water at 37 ° C. In all cases, the dissolution rates of IM from the kneaded mixtures were significantly enhanced, in the order of C-I ≫ C-II > C-III > C-IV ≫ IM. The crystallinity of IM in any kneaded mixture was same as IM particles from analysis of the DSC and X-ray diffraction patterns. Therefore, the observed enhancements in dissolution rate may be due to the increase in solubility as expected from Fig. 1 and the improvement in wettability. These results indicate that LM chitosans, especially C-I, are useful for improvement of the dissolution rate of IM.

In vivo absorption study

The kneaded mixture was expected to have a rapid absorption rate, because of its faster dissolution rate than IM alone. Fig. 8 shows the plasma levels of IM following the oral administration of IM alone or its kneaded mixture to beagle dogs. The bioavailability parameters were calculated from the plasma level-time curves up to 8 h post-administration, and the results are summarized in

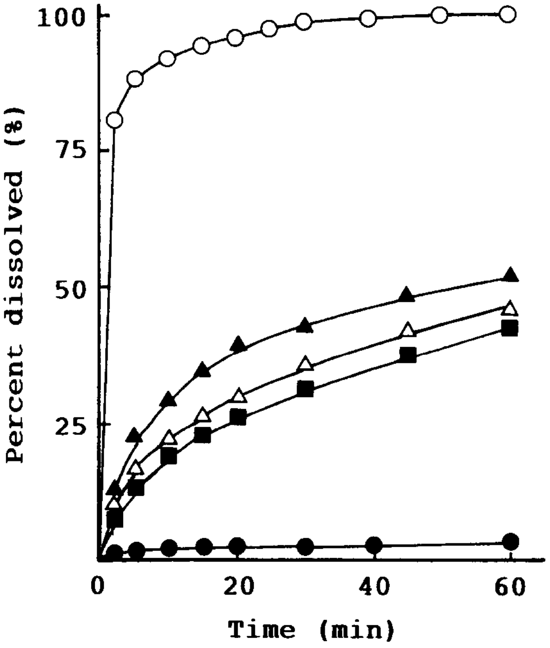

Fig. 7. Dissolution profiles of IM and its kneaded mixtures with LM chitosans in water at 37°C. (●) IM alone; (○) IM-C-I kneaded mixture (1:2 w/w); (▲) IM-C-II kneaded mixture (1:2 w/w); (△) IM-C-III kneaded mixture (1:2 w/w); (■) IM-C-IV kneaded mixture (1:2 w/w). Each point represents the average of 3 experiments.

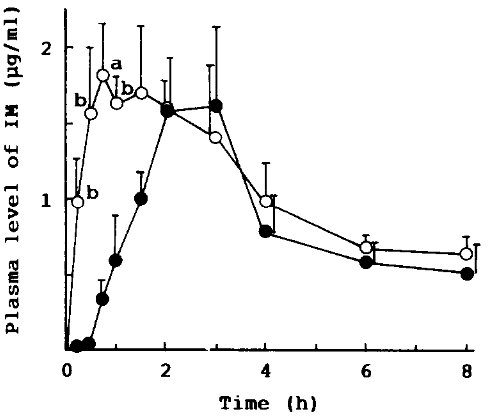

Fig. 8. Plasma levels of IM following the oral administration of IM and its kneaded mixture with C-I (equivalent to 2 mg/kg IM) to beagle dogs. (●) IM alone; (○) IM-C-I kneaded mixture (1:2). Values represent the mean ± S.E. of 4 dogs. (a) $p < 0.01$ in the kneaded mixture vs IM alone; (b) $p < 0.05$ in the kneaded mixture vs IM alone.

TABLE 2

Pharmacokinetic parameters for IM following oral administration of IM and its kneaded mixture with C-I to dogs

	k_a (h^{-1})	$C_{\max}$ (μg/ml)	AUC_{0-8} (μg/ml per h)
IM alone	0.65 ± 0.07	1.93 ± 0.43	6.47 ± 1.53
Kneaded mixture	4.05 ± 1.02 [a]	2.42 ± 0.39	8.57 ± 1.67

Values represent the mean ± S.E. of 4 dogs.

[a] $p < 0.05$ in the kneaded mixture vs IM alone.

Table 2. A significant difference in the absorption rate constant (k_a) was observed between IM and the kneaded mixture. After administration of IM, it scarcely appeared in plasma until 0.5 h, and $T_{\max}$ (time to reach maximum plasma level) was 2–3 h. On the other hand, after administration of the kneaded mixture, the plasma concentration appeared close to maximum plasma concentration ($C_{\max}$) within 0.5 h. No significant differences in $C_{\max}$ and the area under the plasma concentration-time curve (AUC) were observed between IM alone and its kneaded mixture. These data clearly indicated that the faster absorption rate of kneaded mixture was due to the rapid dissolution into the GI fluid.

The present data suggested that LM chitosans, particularly C-I, interact with IM, and are useful for improvement of the dissolution and absorption rates of IM. Thus, the present approach of using a rapidly dissolving form of the C-I kneaded mixture may be promising for improvement of the absorption rate of poorly water-soluble drugs.

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References

Gierasch, L.M., Frey, M.H., Hexem, J.G. and Opella, S.J., Solid state NMR of linear and cyclic peptides. *ACS Symp. Ser.*, 191 (1982) 233–247.

Higuchi, T. and Connors, K.A., Phase solubility techniques. *Adv. Anal. Chem. Instrum.*, 4 (1965) 117–212.

Imai, T., Saito, Y., Matsumoto, H., Satoh, T. and Otagiri, M., Influence of egg albumin on dissolution of several drugs. *Int. J. Pharm.*, 53 (1989) 7–12.

Kaneniwa, N., Otsuka, M. and Hayashi, T., Physicochemical characterization of indomethacin polymorphs and the transformation kinetics in ethanol. *Chem. Pharm. Bull.*, 33 (1985) 3447–3455.

Kawashima, Y., Handa, T., Kasai, A., Takenaka, H., Lin, S.Y. and Ando, Y., Novel method for the preparation of controlled-release theophylline granules coated with a polyelectrolyte complex of sodium polyphosphate-chitosan. *J. Pharm. Sci.*, 74 (1985) 264–268.

Kistenmacher, T.J. and Marsh, R.E., Crystal and molecular structure of an antiinflammatory agent, indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. *J. Am. Chem. Soc.*, 94 (1972) 1340–1345.

Lunazzi, L., Rotational barrier of benzaldehyde as determined by C-13 NMR spectroscopy. *Tetrahedron Lett.*, (1975) 1205–1206.

Miyazaki, S., Ishii, K. and Nadai, T., The use of chitin and chitosan as drug carriers. *Chem. Pharm. Bull.*, 29 (1981) 3067–3069.

Otsuka, M. and Kaneniwa, N., A kinetic study of the crystallization process of noncrystalline indomethacin under isothermal conditions. *Chem. Pharm. Bull.*, 36 (1988) 4026–4032.

Rothwell, W.P. and Waugh, J.S., Transverse relaxation of dipolar coupled spin systems under r.f. irradiation: detecting motions in solids. *J. Chem. Phys.*, 74 (1981) 2721–2732.

Saitô, H., Tabeta, R. and Ogawa, K., High-resolution solid-state ^{13}C NMR study of chitosan and its salts with acid: conformational characterization of polymorphs and helical structures as viewed from the conformation-dependent ^{13}C chemical shifts. *Macromolecules*, 20 (1987) 2424–2430.

Sawayanagi, Y., Nambu, N. and Nagai, T., Dissolution properties and bioavailability of phenytoin from ground mixtures with chitin or chitosan. *Chem. Pharm. Bull.*, 31 (1983) 2064–2068.

Shiraishi, S., Arahira, M., Imai, T. and Otagiri, M., Enhancement of dissolution rates of several drugs by low-molecular chitosan and alginate. *Chem. Pharm. Bull.*, 38 (1990) 185–187.

Singh, S.P., Parmar, S.S., Stenberg, V.I. and Farnum, S.A., Carbon-13 nuclear magnetic resonance spectra of anti-inflammatory drugs: phenylbutazone, oxyphenbutazone and indomethacin. *J. Heterocyclic Chem.*, 15 (1978) 13–16.

Suwelack, D., Rothwell, W.P. and Waugh, J.S., Slow molecular motion detected in the NMR spectra of rotating solids. *J. Chem. Phys.*, 73 (1980) 2559–2569.

Van der Hart, D.L., Earl, W.L. and Garroway, A.N., Resolution in carbon-13 NMR of organic solids using high-power proton decoupling and magic-angle sample spinning. *J. Magn. Reson.*, 44 (1981) 361–401.