Molecular Complex Consisting of Two Typical External Medicines: Intermolecular Interaction between Indomethacin and Lidocaine

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The molecular complex formed between indomethacin (IDM) and lidocaine (LDC), which are typical external medicines, was studied. A thermal analysis, microscopic study and phase solubility technique suggested intermolecular interaction between IDM and LDC. The phase solubility profiles with IDM and LDC were classified as A_L-type, indicating the formation of a 1 : 1 stoichiometric molecular complex. The apparent stability constant (K_S), calculated from the slope and the intercept, was 4478.9 M^{-1}. A molecular ion peak was detected at 592.2 (m/z) from fast-atom bombardment-MS measurements, which was in accordance with the sum of the molecular weight for IDM (M_W: 357.81) and LDC (M_W: 234.38). The changes of IR spectra in the C=O stretching region showed that each intact hydrogen bond network was collapsed in the IDM-LDC system and strong interaction between IDM and LDC formed after their kneading. From the ^{1}H-NMR analyses, it was estimated that the dominant interactive site was the IDM carboxylic acid group which associated with the LDC diethyl amino group non-covalently.

Key words molecular complex; indomethacin; lidocaine; external medicine

Molecular complexes have been studied to improve the physico-chemical properties of drugs for the past half century. Higuchi *et al.* reported that caffeine formed complexes with benzoic acid and various benzoate derivative esters in solution.[1] They attempted the first quantitative evaluation regarding the molecular association responsible for the existence of these complexes and the effect on chemical stability. The complexation of some local anesthetic esters such as benzocaine,[2] procaine,[3] and lidocaine[4] (LDC) with caffeine was also attempted to inhibit the hydrolysis of the ester group on these drugs in aqueous solution. To improve chemical stability and/or increase solubility, such molecular coupling, which is not covalent bonding, has been extensively investigated, for example, riboflavin-nicotinamide,[5] ampicillin-benzaldehyde,[6] and iodine-polyvinyl pyrrolidone.[7]

Indomethacin (IDM), a non-steroidal anti-inflammatory drug (NSAID), has been widely used to treat tendovaginitis, arthritis, and muscle pain as an ointment, cream, gel and adhesive plaster. Since IDM is, however, practically insoluble (*ca.* 2.5 μg/ml) in water, cyclodextrins,[8,9] poly(amidoamine) dendrimers,[10] and several additives[11] have been utilized to improve its dissolution. Furthermore, skin permeation of IDM was enhanced by the complexation of additives with L-menthol[12] or smectite.[13]

LDC topical systems are used to relieve the pain and discomfort associated with herpes zoster virus infections of the skin. Our previous study using Neurometer® demonstrated that the application of a 9% LDC ointment decreased sensory nerve function in 12 volunteers. Nevertheless, pain relief was not achieved efficiently, because the hydrochloride salt of LDC did not sufficiently penetrate the skin tissue.[14] Accordingly, the LDC ointment was reformulated by adding IDM which was expected to heal the inflamed region, because there is no commercially available external medicine consisting of LDC and IDM. In a preliminary study, we obtained a reconstituted viscous paste from IDM and LDC kneaded with an appropriate amount of water.

In this study, the intermolecular interaction between IDM and LDC was examined and the formation of a complex was confirmed using thermal analysis, a polarized microscope with a hot stage, fast-atom bombardment (FAB)-MS, phase-solubility techniques, IR, and ^{1}H-NMR. Finally, the molecular structure of the novel complex in aqueous solution was estimated.

Experimental

Materials IDM and LDC were of reagent grade and purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals and solvents were of reagent grade and used without further purification.

Preparation of the IDM and LDC Complex An equimolar mixture of IDM and LDC was kneaded with an appropriate amount of distilled water using an agate mortar and pestle for 20 min. Then the milled mixture was dried at room temperature under reduced pressure overnight.

Determination of IDM and LDC IDM, LDC, and the kneaded mixture were subjected to a HPLC analysis (LC-2000, Jasco, Tokyo, Japan). The conditions were as follows: mobile phase, methanol–phosphate buffer (pH 6.5) mixture (65 : 35); column, Inertsil ODS-3V (5 μm, 4.6 mm i.d.×150 mm, GL Science Co., Inc., Japan); flow rate 1.0 ml/min; column temperature, ambient; detector, UV at 254 nm.

Thermal Analysis Thermogravimetry (TG) and differential scanning calorimetry (DSC) were carried out with a TG 8230 and DSC 8230 (Rigaku, Tokyo, Japan), respectively. IDM, LDC, and the kneaded mixture were put into an aluminum open pan and measured at a scanning speed of 5 °C min^{-1} under a nitrogen gas flow (100 ml/min) in each thermal analysis.

Polarized Microscopy Thermal behavior was observed optically with an E800 POL polarized microscope (Nikon, Japan) connected to an automatic heater 10002 (Linkam Scientific Instruments Ltd., England). The heating condition was 1 °C min^{-1} from the desired temperature which was 5 °C lower than the predicted melting point, according to JP 15.

Phase Solubility Diagrams Solubility experiments were performed as described by Higuchi and Connors.[15] Approximately 20 mg of IDM was added to various concentrations of LDC in distilled water (0—1.4 mg/ml, 5 ml). The suspensions were shaken in a water bath at 20 °C during 1 week. An aliquot was filtered through a 0.45 μm membrane filter and assayed by HPLC to obtain a phase solubility diagram. The apparent stability constant K_S was calculated from the initial slope and intercept of the phase diagram with Eq. 1.

$$K_S = \frac{\text{slope}}{\text{intercept}(1-\text{slope})} \quad (1)$$

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Infrared Spectroscopy A model 230 FT-IR spectrometer (Jasco, Tokyo, Japan) was used. The measurements were carried out using the KBr method. IDM, LDC, and the kneaded mixture were dried at 25 °C under reduced pressure for 1 d before the measurements.

Mass Specrometry An equimolar mixture of IDM and LDC was added to glycerol and subjected to FAB-MS. The FAB-MS analysis was carried out using a JMS-GCmate mass spectrometer (JEOL, Tokyo, Japan).

^{1}H-NMR The ^{1}H-NMR spectra were measured with a JEOL 600 FT NMR spectrometer (JEOL, Tokyo, Japan). Samples were examined as solutions in D_2O with a 5 mm i.d. sample tube at 30 °C. TMS was used as an external reference to prevent interaction between TMS and IDM or LDC.

Results and Discussion

Thermal Analysis DSC measurements were carried out to clarify the thermal behavior of IDM, LDC and the kneaded mixture as shown in Fig. 1. Two sharp endothermic peaks were observed at 66 and 163 °C which were attributed to the melting of LDC and IDM, respectively. In addition, a broad endothermic peak appeared at around 200 °C in the DSC curves of LDC. TG curves clearly showed the weight loss of LDC in the range of 150 and 220 °C (Fig. 2). Thus, the broad endothermic peak indicated the thermal consumption caused by LDC's vaporization. However, kneaded sample seemed to be sol-gel viscous liquid, and the obvious endothermic peak indicating LDC's melting disappeared (Fig. 1a), and weight loss of LDC was suppressed (Fig. 2b) in the case of IDM/LDC. This suggested that intermolecular interaction between IDM and LDC was generated during the kneading process.

Microscopic Study In order to confirm the phenomena that occurred during the DSC and TG measurements, thermal event was observed using polarized microscope with hot-stage. Figure 3 shows the visual variations of IDM, LDC, and IDM/LDC. The results were well consistent with those of the thermal analysis, that is, melting and vaporization were observed at similar temperatures to those mentioned above in the thermal analysis. When IDM and LDC were melted, crystalline reflections were diminished (Figs. 3b, e). A part of sample vapor adsorbed on the surface of cover glass and crystallized at room temperature within an hour and a few days, respectively (Figs. 3c, f). In contrast, no crystalline particles were observed in the IDM/LDC measurements during storage for at least one month (Fig. 3i). The molecular complex consisting of IDM and LDC might be in a liquid state under ambient conditions or need a long time to crystallize due to its high viscosity.

Phase Solubility Diagram Since a complex was suggested to have formed in the kneading with solid IDM and LDC based on the thermal examination, a phase solubility technique was used to reveal how the molecules interacted in aqueous solution (Fig. 4). The phase solubility profiles with IDM and LDC were classified as being of A_L-type, indicating the formation of a 1 : 1 stoichiometric molecular complex. The apparent stability constant (K_S), which was calculated from the slope and intercept, was 4478.9 M^{-1}, and showed that a water-soluble complex was formed in the concentration range examined. Recently, our research group elucidated the crystal structure of an IDM/LDC complex formed at a 2 : 1 molar ratio, which was crystallized from an ethanol solution.[16] Thus IDM and LDC would interact at a 2 : 1 molar ratio when dissolved in organic solvent at higher concentration than that of aqueous solution. In this case, LDC molecules formed a novel hydrogen bond network which was different from that of both LDC free form[17] and its hydrochloride monohydrate[18] as shown in Fig. 5. Although coprecipitated crystals were not obtained from water in the present study, IDM could behave as an acid instead of hydrochrolic acid and form a complex with LDC in aqueous solution. In addition, the solubility of IDM was 8.6 times greater than that of intact crystalline IDM. Therefore, this formulation was possibly applicable to novel external dosage forms, as the increase in IDM's solubility could enhance the penetration of IDM into skin tissue.

MS Spectrum Stoichiometry was confirmed by using FAB-MS to analyze the molecular weight distribution in the IDM and LDC system. As shown in Fig. 6, the FAB-MS spectrum displays a base peak for LDC and IDM at 235 and 357 (*m*/*z*), respectively. The difference in relative signal intensity between IDM and LDC was speculated to be responsible for the distinction in the ionization efficiency of each compound. A molecular ion peak was detected at 592.2 (*m*/*z*), which was in accordance with the sum of the molecular weight of IDM (M_W: 357.81) and LDC (M_W: 234.38). For most organic molecular complexes, as the energy of attraction between the components of the complex is relatively small (20 kJ/mol),[19] coupled molecules can be separated by their ionization in MS. Consequently, although the intensity of the molecular ion peak was small as a result, IDM and LDC would form a complex at a 1 : 1 molar ratio.

IR Measurement For a structural characterization of the complex, IR spectroscopy was employed. In the IR spectra for both IDM alone and the physical mixture, characteristic bands were observed at 1717 and 1692 cm^{-1}, corresponding to the carbonic stretching band of the γ-form of IDM (Figs. 7a, c). The 1717 cm^{-1} absorption band was assigned to the carbonyl stretch of the carboxylic acid dimer. The 1692 cm^{-1} absorption band was assumed to be the carbonyl stretch of the non-protonated amide.[20] A broad peak was also observed

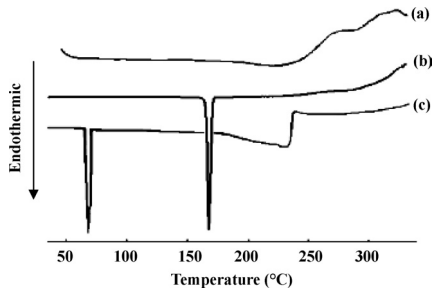


Fig. 1. DSC Thermograms of IDC-LDC Systems, (a) Kneaded Mixture of LDC and IDM, (b) IDM, (c) LDC

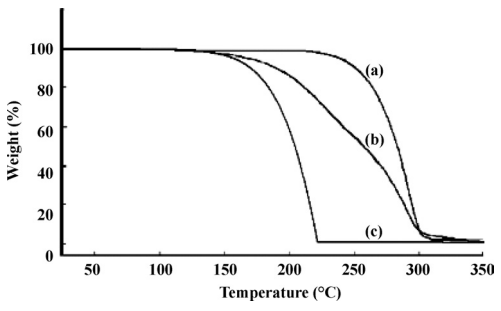


Fig. 2. TG Curves of IDM-LDC Systems, (a) IDM, (b) Kneaded Mixture of LDC and IDM, (c) LDC

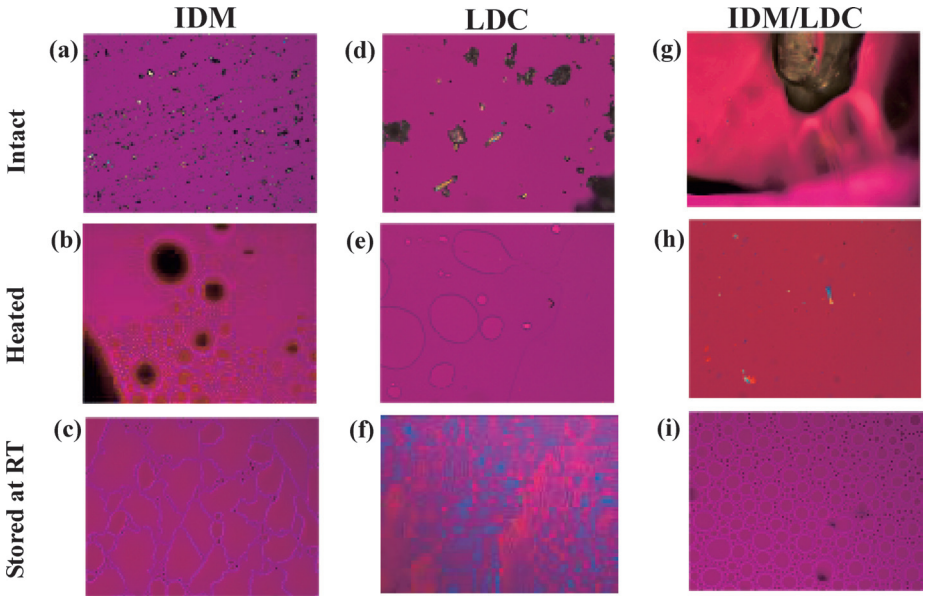


Fig. 3. Hot-Stage Polarized Micrographs of IDM-LDC Systems, (a, d, g) Intact, (b, e, h) Heated, (c, f, i) Observed with Cover Glass

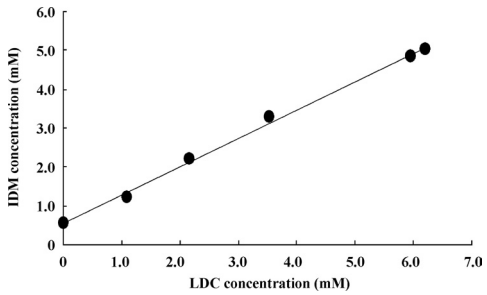


Fig. 4. Phase-Solubility Diagram of the IDM-LDC System

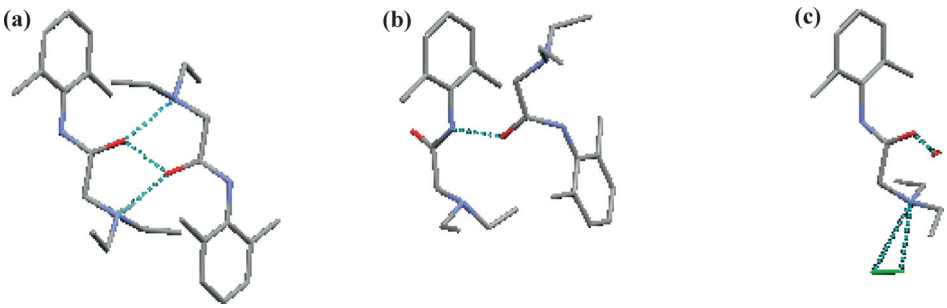


Fig. 5. Crystal Structure of LDC, (a) IDM-LDC Complex,[16] (b) LDC Free Form,[17] (c) LDC Hydrochloride Monohydrate[18]
Blue, red, and green lines represent nitrogen, oxygen and chlorine atoms, respectively. Dashed lines express the hydrogen bonds or intermolecular attractive force.

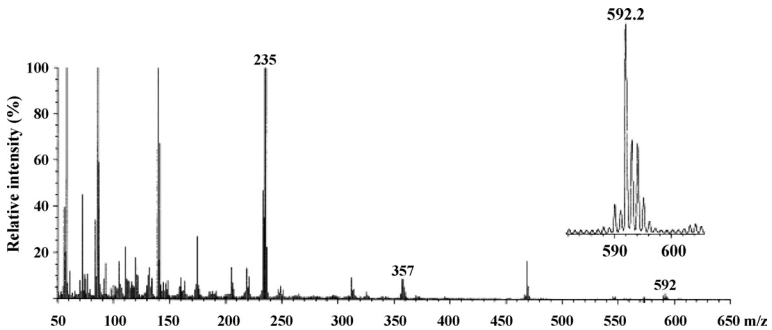


Fig. 6. Mass Spectrum of the Complex Consisting of IDM and LDC

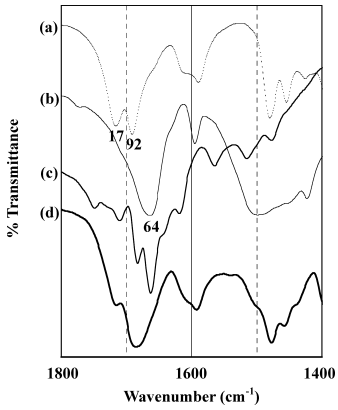


Fig. 7. FT-IR Spectra for IDM-LDC Systems, (a) IDM, (b) LDC, (c) Physical Mixture, (d) Kneaded Mixture of IDM and LDC

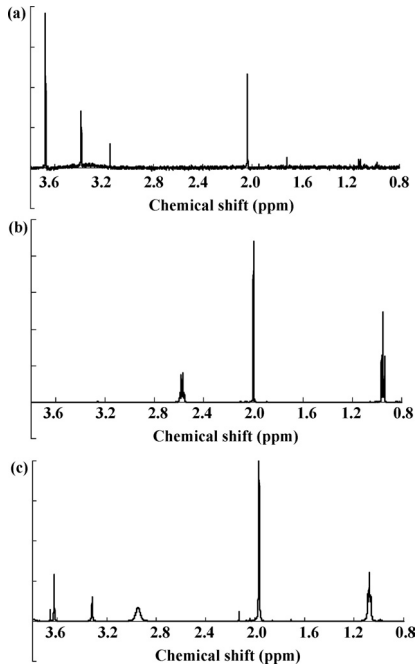
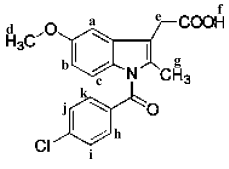
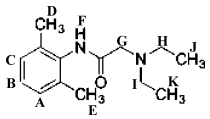


Fig. 8. ^{1}H-NMR Spectra for IDM-LDC Systems, (a) IDM, (b) LDC, (c) Kneaded Mixture of IDM and LDC

at 1664 cm^{-1}, being assigned to the carbonyl stretching vibration of LDC (Figs. 7b, c). After kneading, the carbonyl stretching bands united into a broad peak at around 1685 cm^{-1} in the C=O stretching region. Additionally, a general reduction in the intensity of the bands and a loss of spectral resolution were observed. This indicated that the hydrogen bonding feature in the IDM-LDC system varied from the intact state.

Casella *et al.* reported that complex formed between IDM and β-cyclodextrin, and mentioned two characteristic band changes. One was diminishment of a carbonyl band at 1720 cm^{-1}, the other was a carbonyl band shift from 1690 to 1680 cm^{-1}.[21] On the contrary, in the complexation of LDC with heptakis (2,6-di-*o*-methyl)-β-cyclodextrin (DM-β-CD), the carbonyl stretching bands shifted to a longer frequency, *i.e.* from 1664 to 1686 cm^{-1}, indicating the dissociation of the intermolecular hydrogen bonds of LDC through the complexation.[22] Hence it was suggested that each intact hydrogen bond network collapsed and strong interactions between

Table 1. Proton Chemical Shifts in D_2O at 30 °C (ppm)

		IDM	LDC/IDM	Δd
	g (s)	2.0356	1.9690	−0.0666
	e (s)	3.3890	3.3221	−0.0669
	d (s)	3.6795	3.6250	−0.0545
	b (q)	6.5721	6.4454	−0.1267
	c (d)	6.8904	6.7281	−0.1623
	a (d)	6.9047	6.8139	−0.0908
	h, k (d)	7.4145	7.3230	−0.0915
	i, j (d)	7.5289	7.4032	−0.1258
		LDC	LDC/IDM	Δd
	J, K (t)	0.9501	1.0775	0.1274
	D, E (s)	1.9995	1.9690	−0.0305
	H, I (q)	2.5779	2.9471	0.3692
	G (s)	3.2095	3.6559	0.4464
	A, B, C (t)	7.0080	7.0176	0.0096

Δd=dLDC/IDM-dalone.

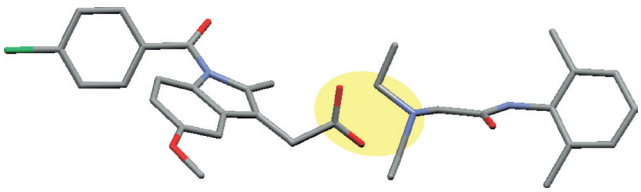


Fig. 9. Speculated Intermolecular Interactive Site between IDM and LDC

IDM and LDC was formed, since both carbonyl absorptions of IDM and LDC showed a similar band shift when interacting with the CD molecule.

^{1}H-NMR The NMR analysis further provided information about the identity of the reaction point. Figure 8 shows ^{1}H-NMR spectra of the IDM-LDC system. When IDM and LDC were mixed at a 1 : 1 molar ratio, ^{1}H chemical shifts were altered as summarized in Table 1. The signals of the methyl and methylene protons belonging to the diethyl amino group of LDC shifted greatly and the peaks of methylene protons broadened. This suggested that the free rotation of methylene protons was inhibited by the other molecule existing around a diethyl amine group of LDC. On complexation, all protons of IDM tended to shift upfield to approximately 0.1 ppm, suggesting an increment of nuclear shielding effect. These results indicated changes in molecular state and/or steric hindrance during the formation of a complex between IDM and LDC.

According to Higuchi *et al.*, the intermolecular interaction of some local anesthesia esters and caffeine could be explained by dipole–dipole interaction, that is, the nitrogen between two carbonyl groups of caffeine became electrophilic and acted like a weak acid.[2,3] Since IDM has a carboxylic acid group, a polarized acidic proton would induce the dipole moment of LDC and interact with the nucleophilic diethyl amino group of LDC (Fig. 9). Further study is expected to elucidate the interactive manner, because other weak intermolecular attractive force such a π–π interaction should be considered.

Conclusion

It is concluded that IDM and LDC formed a water-soluble molecular complex at a 1 : 1 molar ratio in aqueous solution.

The dominant interactive site was estimated as follows: IDM's carboxylic acid group non-covalently associated with LDC's diethyl amino group. Most investigations find an improvement in physicochemical properties in molecular complexes. The complex consisting of IDM and LDC was expected to have a synergistic pharmacological effect. Accordingly, this finding may provide new possibilities in the development of pharmaceutical products.

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