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_{11}-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 $4478.9 \text{ M}^{-1}$. 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 formed between IDM and LDC 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

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 high 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 mixed 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. x 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 heated stage (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.\textsuperscript{10} 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.

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

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technique was used to reveal how the molecules interacted in gestures to have formed in the kneading with solid IDM and crystallize due to its high viscosity. A liquid state under ambient conditions or need a long time to melt, crystalline reflections were diminished (Figs. 3b, e). In contrast, no crystalline reflections 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 A₂-type, indicating the formation of a 1 : 1 stoichiometric molecular complex. The apparent stability constant (Ks), 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. 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 and its hydrochloride monohydrate 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 hydrochloric 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.

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 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 the kneaded mixture of IDM/LDC. The apparent stability constant (Ks), 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. 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 and its hydrochloride monohydrate 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 hydrochloric 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), 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 carbonyl 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. A broad peak was also observed in aqueous solution (Fig. 4).
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, (b) LDC Free Form, (c) LDC Hydrochloride Monohydrate
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
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 \(\beta\)-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\(^{-1}\). On the contrary, in the complexation of LDC with heptakis (2,6-di-o-methyl)-\(\beta\)-cyclodextrin (DM-\(\beta\)-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. Hence it was suggested that each intact hydrogen bond network collapsed and strong interactions between IDM and LDC was formed, since both carbonyl absorptions of IDM and LDC showed a similar band shift when interacting with the CD molecule.

\(\text{\(^1\)H-NMR}\) The NMR analysis further provided information about the identity of the reaction point. Figure 8 shows \(\text{\(^1\)H-NMR}\) spectra of the IDM-LDC system. When IDM and LDC were mixed at a 1:1 molar ratio, \(\text{\(^1\)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.

Table 1. Proton Chemical Shifts in D\(_2\)O at 30 °C (ppm)

<table>
<thead>
<tr>
<th>Proton</th>
<th>IDM</th>
<th>LDC/IDM</th>
<th>(\Delta d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g (s)</td>
<td>2.0356</td>
<td>1.9690</td>
<td>-0.0666</td>
</tr>
<tr>
<td>e (s)</td>
<td>3.3890</td>
<td>3.3221</td>
<td>-0.0669</td>
</tr>
<tr>
<td>d (s)</td>
<td>3.6795</td>
<td>3.6250</td>
<td>-0.0545</td>
</tr>
<tr>
<td>b (q)</td>
<td>6.5721</td>
<td>6.4454</td>
<td>-0.1267</td>
</tr>
<tr>
<td>c (d)</td>
<td>6.8904</td>
<td>6.7281</td>
<td>-0.1623</td>
</tr>
<tr>
<td>a (d)</td>
<td>6.9047</td>
<td>6.8139</td>
<td>-0.0908</td>
</tr>
<tr>
<td>h, k (d)</td>
<td>7.4145</td>
<td>7.3230</td>
<td>-0.0915</td>
</tr>
<tr>
<td>i, j (d)</td>
<td>7.5289</td>
<td>7.4032</td>
<td>-0.1258</td>
</tr>
</tbody>
</table>

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.

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. 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 as \(\pi-\pi\) 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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References