Particle Condition Change in Emulsion Admixture Evaluated by in Situ Flow Particle Imaging Analysis

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We evaluated the particle state change in emulsion admixtures using in situ flow particle imaging analysis (FPIA). Ropion® intravenous (flurbiprofen axetil: Ropion®) served as the model emulsion formulation. A binary mixture of Ropion® and normal saline (NS), and a ternary admixture of Ropion®, NS, and Gaster® injection (famotidine: Gaster®) or Primperan® injection (metoclopramide hydrochloride: Primperan®) were prepared and the change in emulsion particle state was analyzed using FPIA under in situ condition. The effect of storage on pH change and the chemical stability of flurbiprofen axetil were also investigated. In Ropion®, various particle images (mean diameter: 2.4µm) were obtained. From our analysis of changes in scattergrams and particle images, changing behaviors of emulsion admixtures as a function of storage time depended on the systems of admixture samples. In Ropion®/NS and Ropion®/Gaster®/NS systems, mean particle size and particle number increased with lengthening storage time; however, these values were dramatically increased beyond 6h in the Ropion®/Primperan®/NS system, corresponding to a decrease in measured pH. The decomposition of flurbiprofen axetil due to incompatibility was not observed in all systems. Detailed information on the change in emulsion particle state was obtained using FPIA, indicating that this method is useful to evaluate state changes in emulsion admixtures under in situ condition.

Key words flow particle imaging analysis; incompatibility; emulsion; Ropion® intravenous; flurbiprofen axetil

Parenteral injections have the advantages of both immediate systemic drug availability and rapid onset of action. When an injectable drug is administered, it is usually prepared at an appropriate dose for a specific patient. The preparation process (dilution and dissolution of a drug in an infusional diluent) can result in physicochemical incompatibility and affect the stability of the drug.1–3)

Intravenous emulsions are used in the delivery of both nutritional supplements and in the preparation of drugs with low water solubility.4) The admixing of an intravenous emulsion with another component is not recommended by manufacturers because it usually results in instability of the emulsion.5) However, in clinical practice, admixtures of intravenous emulsions with other components are often prepared in order to reduce the number of daily injections, and certain reports have described the co-infusion of intravenous emulsions with other injected drugs.6)

The stability of intravenous emulsions is likely to be related to their pharmacokinetics. The chemical dimension determines the stability of a drug’s medicinal properties, and thus is an important property in terms of treatment. Variations in emulsion formulations belong to the physical dimension. These features are indirectly related to the effects of drug treatment, unlike those in the chemical dimension. Changes in the physical dimension include the degradation of the emulsion through altered particle size, surface, and composition properties. These types of changes might result in alterations in the pharmacokinetics.7) Therefore, it is important to evaluate the physicochemical stability of emulsion preparations.

In general, the physical stability of emulsion particles is estimated by mean particle size and zeta potential using dynamic light scattering analysis.8–10) Thus, it is difficult to evaluate intact samples in situ, because these measurements require the samples to be diluted for analysis. Flow particle imaging analysis (FPIA) is used in the measurement of various particles: microbial cells, toner particles, denatured proteins, and injected particles.12–16) FPIA has a high detection throughput, and is therefore well-suited for estimating particle size in situ in turbid solutions such as suspensions and emulsions. Additionally, it can provide an assessment of not only particle number, but also particle size and shape by generating images of samples. Its ability to provide data on multiple variables in turn creates a better understanding of various particle behaviors. Thus, we propose that FPIA is a useful method for the evaluation of emulsion stability in situ.

There are no previous reports in the literature describing analyses of the in situ physicochemical compatibility of intravenous emulsion materials. In this study, Ropion® intravenous 50mg (Ropion®)—an intravenous emulsion including flurbiprofen axetil—was selected as a model intravenous emulsion. This intravenous emulsion is used as an analgesic for postoperative or carcinomatous pain.17,18) Gaster® injection 20mg (famotidine: Gaster®) and Primperan® injection 10mg (metoclopramide hydrochloride: Primperan®) were used as admixture injections with Ropion®. General information of these injections was summarized in Table 1.

These medications are commonly used in clinical practice. Information regarding the incompatibility of Ropion® and other injected drug preparations have been summarized by manufacturers and include measurements of particle size change using dynamic light scattering analysis (Kaken

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Pharmaceutical Co.). The compatibility of Ropion® with Gaster® and Primperan® is including in this report.

In the present study, an intact Ropion® emulsion was evaluated by FPIA. A binary admixture of Ropion® with normal saline (NS), and a ternary admixture of Ropion®, NS, and either Gaster® or Primperan® was then prepared, and the emulsion stability was investigated by FPIA. Moreover, the chemical stability of flurbiprofen axetil in the admixture, as well as pH changes of the admixture as a function of storage duration were also evaluated.

**Experimental**

**Materials** Ropion® intravenous 50 mg (Kaken Pharmaceutical Co., Ltd., solution volume: 5 mL) was used as a model intravenous emulsion. Gaster® injection 20 mg (Astellas Pharma Inc., solution volume: 2 mL), Primperan® injection 10 mg (Astellas Pharma Inc., solution volume: 2 mL), and normal saline solution (Otsuka Pharmaceutical Co., Ltd., solution volume: 50 mL) were used for compatibility testing with Ropion®. Flurbiprofen axetil was used as an internal standard and was kindly supplied by Kaken Pharmaceutical Co., Ltd., Japan. Other chemicals and solvents were of reagent grade and were used without further purification.

**Sample Preparation** Admixtures for the compatibility test were prepared by the dilution of each injection with 50 mL NS. In the ternary admixture, 2 different drug combinations were contained in NS. All samples were prepared under sterile conditions; pharmacists prepared the injectable drugs using a disposable polypropylene syringe and needle.

**Physicochemical Compatibility Tests of Admixtures after Preparation** Compatibility tests were performed for each of the prepared admixtures. Prepared samples were stored in the clean bench at 25°C. At specific time intervals, 5 mL samples were obtained from each admixture and were analyzed by FPIA and HPLC. For HPLC analysis, flurbiprofen axetil was extracted from the admixture samples using 2-propanol and the samples were diluted to 100 times. Detailed experimental conditions for FPIA and HPLC are described below.

**pH Measurement** The pH of the samples was measured by pH Spear (Eutech Instruments Europe B.V., Nijkerk, the Netherlands). Measurements were performed by soaking the electrode in the prepared injectable drugs after calibration using pH 4.0 and 9.0 standard solutions.

**FPIA Measurements** The Sysmex FPIA-3000 (Malvern Instruments Japan, Hyogo, Japan) can provide both particle size and shape distribution data. Samples (5 mL) were passed through the measurement aperture where images were captured using stroboscope illumination and a charge coupled device (CCD) camera. Digital images of particle samples were collected, and multiple parameters of each particle were calculated automatically by the FPIA-3000 software. Particle size was evaluated by calculating the diameter of a circle with the same cross-sectional area as the particle (CE diameter). The shape of particles was determined by their circularity (Ç), which is the ratio between the circumference of a circle with the same area as the projected area of the particle image (S), and the circumference of the particle image (T) (Ç=S/T). To obtain precise values, the Sysmex FPIA-3000 was calibrated before conducting experiments by use of a certified size standard, consisting of an aqueous suspension of polymer microspheres with a diameter of 2 μm. Samples were prepared, and then tested in triplicate at each time point using both high- and low-power field modes. The high-power field (HPF) mode permits measurement of particles with an effective size range between 1.5–40 μm, whereas the low-power field (LPF) mode provides a size range between 8–160 μm (values that are over or under the detection limit are displayed as gray regions in the scattergram). The detection count limit was 360000/2 min.

**HPLC Analysis** The HPLC system consisted of a pump (LC-10AT; Shimadzu Co., Ltd., Kyoto, Japan), a column (Synergi 4u Fusion-RP 80A Packed Column, 4.6×150 mm; Phenomenex Inc., Torrance, CA, U.S.A.), an auto-injector (SIL-10AXL, Shimadzu Co., Ltd., Tokyo, Japan), a UV detector (SPD-10A, Shimadzu Co., Ltd., Tokyo, Japan), and an analysis system (CLASS LC-10, Shimadzu Co., Ltd., Tokyo, Japan). The mobile phase was used in the appropriate ratio of 50 mM phosphate buffer solution–methanol (20:80). The flow rate was 1.0 mL/min. Detection was performed at a UV wavelength of 254 nm. Flurbiprofen axetil standard solutions were found to be linear at concentrations of 25–800 μg/mL. The concentration was determined from the averaged value measured from triplicate samples with a relative standard deviation.

**Results and Discussion**

To obtain particle data from the Ropion® emulsion, the injection was directly analyzed by FPIA. Figure 1 shows data generated using FPIA in HPF mode, including the particle size cumulative frequency curve, the scattergram (circularity vs. CE diameter), and selected particles of intact Ropion® observed within the indicated colored regions of the scattergram.
From the results of the analysis shown in Fig. 1a, the average median CE diameter of Ropion® was 2.4 µm. A large number of particles was observed and their data are summarized in the scattergram (circularity vs. CE diameter) (Fig. 1b). Fine emulsified particles (particle size >1.5 µm, circularity >0.9) were observed in the green region of Fig. 1b (Fig. 1c). In the orange square region of Fig. 1b, various types of particle images (multiple or fused particles) were obtained (particle size: 1.5–10 µm, 0.2 < circularity < 0.9) (Fig. 1e). In the lower circularity (<0.9) and larger particle size (>10 µm) region surrounded by the red square, aggregated or very large single particles were observed (Fig. 1d). From the results of the analysis shown in Fig. 1, it was found that detailed particle information about Ropion® emulsion can be obtained by FPIA measurements under in situ condition.

Although intravenous administration of Ropion® are specified in its accompanying package insert, continuous intravenous drips of Ropion® are often used with a saline dilution. As we mentioned previously, Ropion® can be prepared and administered as either a binary or ternary admixture in clinical practice. Therefore, it is important to evaluate emulsion stability after dilution in saline or the addition of another drug (Gaster® and Primperan®). Figure 2 shows the effect of storage of prepared injection on particle stability.

We observed no significant change in visual evaluation for any of the tested samples during the compatibility test (data not shown). At the initial time point, no change in either mean particle size or circularity was observed in either the binary (Ropion®/NS) or ternary samples (Ropion®/Gaster®/NS), compared to Ropion® alone (data not shown). When performing FPIA in HPF mode on the binary system (Ropion®/NS) and the ternary system (Ropion®/Gaster®/NS), a rapid increase in particle numbers was observed at the 0–1 h time point (Fig. 2a). At 6 h after preparation, the particle number measured in HPF mode reached a total of 6635±111 and then decreased. The CE diameters were shown to be increased with longer storage times (Fig. 2b). The particle number measured in LPF mode also increased with lengthening storage times (Fig. 2c). From results of Figs. 2a and b, it might be assumed that change to larger particles beyond the limit of HPF mode (>40 µm) was occurred by the fusion of emulsion particles from 6 h after preparation. The increase of particle number in LPF result (Fig. 2e) might also support the results of Fig. 2a. Therefore, these results suggested that physicochemical incompatibilities with storage were occurred by the preparation of the admixture between Ropion® and another components.
In the ternary sample (Ropion®/Primperan®/NS), different compatibility behavior was observed. The results are shown in Fig. 3.

Within the initial 3 h period after preparation, no significant changes in particle number were observed in either LPF or HPF modes. From 6 h after preparation, however, particle numbers were dramatically increased (Figs. 3a, c) and then reached a plateau. In HPF mode, an increase in CE diameter was also observed (Fig. 3b).

We also compared the compatibility of 2 ternary systems, Ropion®/Gaster®/NS and Ropion®/Primperan®/NS, and the resulting scattergrams (Circularity vs. CE diameter) are shown according to storage time in Fig. 4.

Combined with the result of Fig. 2a, increased and decreased of particle numbers of various circularity were observed with greater storage time in the Ropion®/Gaster®/NS system (Figs. 4a–d). However, lower circularity particles (0.2 < circularity < 0.9) were dramatically increased from 6 h after preparation in the Ropion®/Primperan®/NS system (Figs. 4e–h). Selected particle images observed in the Ropion®/Primperan®/NS samples 24 h (red square region of Fig. 4h) after preparation are shown in Fig. 5.

A large number of images of heteromorphic particles (fused, aggregated, and ruptured) was obtained. Although the particle numbers in Ropion®/Primperan®/NS system remained constant from the 6 h time point (Fig. 3a), Figs. 4 and 5 show that the emulsion conditions between 6 and 24 h after preparation were clearly different in Ropion®/Primperan®/NS system. These results suggest that the incompatibility mechanisms occurring in the 2 systems were different.

In a study of drug incompatibility chemistry, Nemec et al. reported that changes in pH of the admixture after preparation is an important factor affecting stability. Since intravenous emulsions are sensitive to changes in the emulsion interface, a change in pH of the admixture can facilitate degradation. Consequently, emulsion degradation may cause chemical degradation of the incorporated drugs.

Therefore, we investigated the effect of pH change on degradation of flurbiprofen axetil after preparation, by measuring both the percentage of residual drug, as well as pH in our admixtures. Table 2 summarizes our findings of the residual percentage of flurbiprofen axetil, and the pH of our admixtures as a function of the storage time after preparation.

Chemical degradation of flurbiprofen axetil was not observed in either system, suggesting that this drug remained chemically stable in these admixtures. The initial pH of the Ropion®/Gaster®/NS, and the Ropion®/Primperan®/NS system was 5.6 and 4.7, respectively. In the Ropion®/Gaster®/NS system, no pH changes were observed over a period of 24 h. The pH values decreased in the ternary mixture of Ropion®/Primperan®/NS with increasing storage time, and the measured value after 24 h was 3.8. These results indicate that pH change over time in the Ropion®/Primperan®/NS system was an important trigger of the significant change in emulsion conditions. In the Ropion®/Primperan®/NS system, metochrompramide, which had amino group (pK_a=9.1), exist in charged
Fig. 3. Change in the Number of Selected Particles and CE Diameter (μm) as a Function of Storage Time after Preparation of a Ternary System of Ropion®/Primperan®/NS

(a) The number of selected particles and (b) mean particle size (μm) in HPF detection mode. (c) The number of selected particles measured and (d) mean particle size (μm) in LPF detection mode. The samples were measured at 0, 1, 3, 6, 12, and 24 h after preparation. Each data point represents the average of 3 measurements; error bars represent ± S.D.

Fig. 4. Effect of Storage Time after Preparation on Particle Scattergrams (Circularity vs. CE Diameter) in Ropion®/Gaster® or Primperan®/NS Systems

Samples were measured at 0 h (a, e), 6 h (b, f), 12 h (c, g), and 24 h (d, h). Scattergrams from (a)–(d) and from (e)–(h) show either the Ropion®/Gaster® or Primperan®/NS systems, respectively.
molecule and Primperan® injection show low pH from Table 2. These might affect the emulsion stability in the Ropion®/Primperan®/NS system. In manufacturer’s tests, apparent changes in Ropion®/Gaster®/NS and Ropion®/Primperan®/NS occurred at 6 and 1 h post-preparation, respectively. However, no changes in particle size in these systems were reported. The composition of all admixtures described in our results, and those described in the manufacturer’s tests were not identical. The manufacturer’s compatibility testing was conducted using condensed conditions in comparison to our study. Dynamic light scattering analysis has historically been used by drug manufacturers to investigate emulsion particle size during compatibility testing of combinations of Ropion® with other injectable drugs. Therefore, conclusions based on our results, versus those of the drug manufacturer, may differ regarding the incompatibility of intravenous emulsions.

FPIA is capable of measuring in situ admixture samples, in addition to a large range of particle diameters when compared with dynamic light scattering analysis; thus, FPIA has the capacity to evaluate changes in emulsion particles. Additionally, it provides investigators with multilateral information, particle number, diameter, and circularity, and permits discovery of new phenomena related to emulsion particles. Therefore, conclusions based on our results, versus those of the drug manufacturer, may differ regarding the incompatibility of intravenous emulsions.

Table 2. Change of Chemical Stability of Flurbiprofen Axetil and pH as a Function of Storage Time for Ternary Admixtures

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Ropion®+Gaster®+NS</th>
<th>Ropion®+Primperan®+NS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative residual percentage of flurbiprofen axetil (%)</td>
<td>pH</td>
</tr>
<tr>
<td>0</td>
<td>100±1.1</td>
<td>5.6</td>
</tr>
<tr>
<td>1</td>
<td>99.6±1.1</td>
<td>5.6</td>
</tr>
<tr>
<td>3</td>
<td>100.6±0.9</td>
<td>5.6</td>
</tr>
<tr>
<td>6</td>
<td>100.1±0.3</td>
<td>5.6</td>
</tr>
<tr>
<td>12</td>
<td>97.8±0.9</td>
<td>5.6</td>
</tr>
<tr>
<td>24</td>
<td>102.3±0.7</td>
<td>5.6</td>
</tr>
</tbody>
</table>

All data represent the relative residual ratio of flurbiprofen axetil, which was calculated using the amount measured from the 0 h time point as 100%. Each data point represents the average of three measurements±S.D.

As we described in introduction, multiple injection could be administrated to the patients depending on their condition. Therefore, another kind of incompatibility such as crystallization of the injection component in the administration route might be occurred. FPIA might be one of the important tools to evaluate the incompatibility due to crystallization.

**Conclusion**

The compatibility of Ropion® with other injectable drugs in admixture samples was evaluated using the FPIA method. Even in a dilution of Ropion® with a NS solution, changes in particle size and number were observed. In ternary samples consisting of a Ropion®/Primperan®/NS system, FPIA analysis enabled the evaluation of changes in particle states due to increasing storage time after preparation. Thus, FPIA was strongly indicated as a useful tool to evaluate the incompatibility of emulsion formulations under in situ condition.

**Acknowledgment**

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**References**