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Pharmaceutical quality evaluation of lipid emulsions containing PGE_1: Alteration in the number of large particles in infusion solutions

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ABSTRACT

There are two generics of a parenteral lipid emulsion of prostaglandin E1 (PGE_1) (Lipo-PGE_1) in addition to two innovators. It was reported the change from innovator to generic in clinical practice caused the slowing of drip rate and formation of aggregates in the infusion line. Thus, we investigated the difference of pharmaceutical quality in these Lipo-PGE_1 formulations. After mixing with some infusion solutions, the mean diameter and number of large particles were determined. Although the mean diameter did not change in any infusion solutions, the number of large particles (diameter >1.0 μm) dramatically increased in generics with Hartmann's solution pH 8 or Lactec® injection with 7% sodium bicarbonate. Next, we investigated the effect of these infusion solutions on the retention rate of PGE_1 in lipid particles. The retention rate of PGE_1 in these two infusion solutions decreased more quickly than that in normal saline. Nevertheless, there were no significant differences among the formulations tested. Our results suggest that there is no difference between innovators and generics except in mixing with these infusion solutions. Furthermore, that monitoring the number of large particles can be an effective means of evaluating pharmaceutical interactions and/or the stability of lipid emulsions.

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1. Introduction

Prostaglandin E1 (PGE_1), which has a strong vasodilatory and antiplatelet activity, is clinically used to treat diseases such as periferal arterial occlusive diseases (Makita et al., 1997; Milio et al., 2003) and ductus arteriosus-dependent congenital heart disease (Kramer et al., 1995). However, PGE_1 has a very short half-life in blood and can elicit various side-effects (Golub et al., 1975; Schramek and Waldhauser, 1989). As a result, a lipid emulsion of PGE_1 (Lipo-PGE_1), in which lipid particles incorporating PGE_1 were coated with lecithin, was developed and applied for clinical treatment in Japan (Mizushima et al., 1983; Otomo et al., 1985). Because the lipid particles are efficiently distributed into the vascular lesion site, Lipo-PGE_1 accumulates in the lesion area and is therefore safer and more effective than free PGE_1 (Mizushima et al., 1990; Mizushima et al., 1983). Indeed, Lipo-PGE_1 is widely used to treat a number of conditions other than arterial occlusive diseases, such as cutaneous ulcer with diabetes and improvement of imaging ability for arterial portography. Two innovator formulations and two generic formulations have already been launched. The composition of each formulation is shown in Table 1. Although generic formulations contain olive oil instead of soybean oil, the other additives are the same as those found in the innovator formulations. Hydrochloric acid or sodium hydrate is added appropriately as a pH adjuster, and the pH of each formulation is adjusted 4.5–6.0.

Lipo-PGE_1 can be intravenously administered by bolus injection, or slowly administered as infusions by mixing with infusion solution. Recently, it was reported that the change from innovator to generic formulation in clinical practice caused the slowing of drip rate and formation of aggregates in the infusion line (Sakaya et al., 2005; Goto et al., 2005). This phenomenon was observed under alkaline conditions in the presence of calcium ions. The Lipo-PGE_1 has an approximate pH of 5. There are some cases where Lipo-PGE_1 is mixed into the infusion solutions of relatively high pH (e.g., Hartmann's solution pH 8; 7% sodium bicarbonate) in order to moderate vascular pain or venous inflammation. Furthermore, it has also been reported that generic formulations in saline solution exhibit lower retention rates of PGE_1 in lipid particles and weaker pharmacological activity in animal models than innovator formulations (Takenaga et al., 2007). Therefore, it is important to investigate the difference in pharmaceutical quality between innovator and generic formulations.

In the Japanese Pharmacopoeia, the diameter of lipid particles in a lipid emulsion is defined as being below 7 μm. Included in the tests for the preparation of a parenteral lipid emulsion is "Insoluble Particulate Matter Test for Injections" as well as "Test for Extractable Volume of Parenteral Preparations". The former test defines an examination by "Method 1. Light Obscuration Particle Count Test" or

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Table 1
Formulas of Lipo-PGE_1.

	Alprostadil (PGE1)	Purified soybean oil	Purified olive oil	Highly purified soybean lecithin	Oleic acid	Concentrated glycerin
Formulation #1 (innovator)	5 μg	100 mg		18 mg	2.4 mg	22.1 mg
Formulation #2 (innovator)	5 μg	100 mg		18 mg	2.4 mg	22.1 mg
Formulation #3 (generic)	5 μg		100 mg	18 mg	2.4 mg	22.1 mg
Formulation #4 (generic)	5 μg		100 mg	18 mg	2.4 mg	22.1 mg

"Method 2. Microscopic Particle Count Test". Method 1 is preferably applied to injections and parenteral infusions. However, in cases where the preparation has a reduced clarity or increased viscosity, such as emulsions, colloids and liposomal preparations, the test should be carried our according to Method 2. The Pharmacopoeia of the United State of America (USP), ⟨788⟩ also defines a similar method for parenteral preparations. However, within recent years, ⟨729⟩ "Globule Size Distribution in Lipid Injectable Emulsions" is listed in the second supplement of USP30. This ⟨729⟩ provides two methods, "Method 1. Light-Scattering Method" for the mean diameter of lipid particles, and "Method 2. Measurement of Large Globule Content by Light Obscuration or Extinction method" for the extent of large-diameter particles (>5 μm), and is required to meet both criteria. This is based on the idea that the size of the lipid particles is critical because large-size fat globules can become trapped in the smallest of blood vessels such as capillaries with diameters between 4 and 9 μm (Guyton, 1991). The essential size characteristics of a lipid injectable emulsion include the mean diameter of lipid particles and the range of the various particle diameters distributed around the mean diameter (Driscoll et al., 2001). In this study, we investigated the formation of aggregates and measured the mean diameter and/or number of large-diameter particles. We also monitored PGE_1 retention rate in Lipo-PGE_1 to investigate the difference in the pharmaceutical quality of Lipo-PGE_1 formulations.

2. Materials and methods

2.1. Materials

Four Lipo-PGE_1 formulations as shown in Table 1 were used in this study. Palux® injection (Formulation #1, lot nos. O17H2 and I07H2, Taisho Pharmaceutical Co., Ltd., Tokyo, Japan), Liple® injection (Formulation #2, lot nos. P625J and P205H, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan), Alyprost® injection (Formulation #3, lot nos. AB07A and AF07A, Nippon Chemiphar Co., Ltd., Tokyo, Japan), Prink® injection (Formulation #4, lot nos. 659109 and 659123, Taiyo Yakuhin Co., Ltd., Nagoya, Japan) were purchased from a drug seller in Japan. Otsuka normal saline, Aminofluid®, Lactec® injection and Meylon® (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), Amicaliq® (Termo Corporation, Tokyo, Japan), Solita®-T No. 3 (Ajinomoto Co., Inc., Tokyo, Japan), Hartmann's Solution pH 8 and Nipro infusion set IS type (Nipro Pharma Corporation, Osaka, Japan), were purchased from a general sales agency for drugs in Japan. Lactec® injection with $NaHCO_3$ was composed of 500 ml Lactec® injection and 20 ml Meylon® (7% $NaHCO_3$ injection). The official PGE_1 reference standard was purchased from the Society of Japan Pharmacopoeia. Slide-A-Lyzer ® Dialysis Cassette (molecular weight cutoff: 7 K, capacity: 0.1–0.5 ml) and Buoy used for dialysis method were purchased from Pierce (IL, US). Disposable syringes, 21- and 27-gauge needles were purchased from Terumo Corporation.

2.2. Particle size distribution analysis

A 2 ml aliquot of Lipo-PGE_1 was injected into a 500 ml infusion bag of different solutions. After mixing, the various solutions were incubated at room temperature. At the indicated time point, each mixed solution was collected and analyzed by measurement of dynamic light scattering or single particle optical sizing.

2.2.1. Dynamic light scattering (DLS)

The particle size distribution and mean diameter of each Lipo-PGE_1 after mixing with different solutions were measured using a dynamic light scattering photometer DLS-7000 (Otsuka Electronics Co., Ltd., Osaka, Japan) equipped with a He–Ne laser source (wavelength, 632.8 nm). All DLS measurements were made with a scattering angle of 90°. Mixed solutions were diluted 15-fold with each infusion solution in order to obtain an appropriate scattering intensity. Data were gathered using a counting period of 100 s. Histogram analyses were performed to calculate the average particle size and standard deviation.

2.2.2. Single particle optical sizing (SPOS)

An Accusizer 780A (Particle Sizing Systems, Santa Barbara, CA) was used to determine the number of large-diameter particles in the emulsions. This instrument is based on light extinction (LE) or light scattering (LS) that employs a single-particle optical sizing (SPOS) technique, and was equipped with an automatic dilution system. In this study, the summation mode, which is a combination of LE and LS, was applied to measure the number of particles >0.5 μm in diameter. Before commencing any measurements, the equipment was filled with each infusion solution by using the command "Start Vessel Flush". After ensuring the background count was below 100 counts/s, mixed solutions (about 5 ml) were injected into the sample chamber. Duplicate measurements were made for each sample at the appropriate time point using the following conditions; data collecting time, 60 s; flow rate, 60 ml/min; injection loop volume, 1.04 ml; syringe volume, 2.5 ml; second dilution factor, 40. We ascertained that this dilution factor maintained the per-milliliter counts below the coincidence limit for the sensor, thereby minimizing this source of error. The volume-weighted proportion of fat globules (PFAT) with a diameter of >5 μm ($PFAT_5$) was calculated by the command "Volume Fraction cal".

2.3. Zeta potential

Zeta potential was measured using a Zetasizer NanoZS (Malvern Instruments, Malvern, UK), which is based on laser Doppler velocimetry in an electric field. For each Lipo-PGE_1, 500 μl was diluted using 10 ml distilled water.

2.4. Determination of PGE_1 retention rate

2.4.1. Assay for PGE_1

PGE_1 was measured by high-performance liquid chromatography (HPLC) using a post-column reaction. The HPLC system consisted of two constant pumps (LC-10ADvp, Shimadzu, Kyoto, Japan), a degasser (DGU-14A, Shimadzu), an automated pretreatment system, an autoinjector (SIL-10ADvp, Shimadzu), a UV/VIS detector (SPD-20AD, Shimadzu), a column oven (CTO-10ACvp, Shimadzu), and a system controller (SCL-10Asp, Shimadzu). PGE_1 was detected at 278 nm. The column used in this study was a 15 cm stainless-steel (4.6 mm i.d.) 5 μm Ø Mightysil ODS (Kanto Chem-

Table 2
Compositions of infusion solution.

	g/500 ml												
	NaCl	KCl	$CaCl_2$	$MgCl_2$	$C_3H_5NaO_3$	$C_{12}H_{22}CaO_{14}$	K_2HPO_4	$MgSO_4$	ZnSO4	Amino acids	Glucose	pH	mequiv./L
Normal saline	4.50	–	–	–	–	–	–	–	–	–	–	6.0	1
AMINOFLUID®	0.39	–	–	–	1.15	0.41	0.56	0.31	0.70	15.00	37.50	6.7	3
AMICALIQ®	–	0.82	–	0.15	1.41	0.13	–	–	–	13.75	37.50	4.6–5.6	3
SOLITA®-T No. 3	0.45	0.75	–	–	1.12	–	–	–	–	–	21.5 0	3.5–6.5	1
Hartmann's solution pH 8	3.00	0.15	0.10	–	1.55	–	–	–	–	–		7.8–8.2	1
Lactec® Injection	3.00	0.15	0.10	–	1.55	–	–	–	–	–		6.0–8.5	0.9
MEYLON®	$NaHCO_3$	1.4 g/20 ml										7.0–8.5	5

ical Co., Inc., Tokyo, Japan) with a 10 m Teflon tube (0.5 mm i.d.) as a post-column. The automated pretreatment system consisted of a pretreatment column, a constant pump for cleaning solutions, and two switching valves. The pretreatment column used was a 2.5 cm stainless-steel (4.0 mm i.d.) 5 μm Ø LiChroCART 25-4 Lichrospher 100RP-18e (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile:1/150 M phosphate-buffered solution (pH 6.3). The ratio of organic to aqueous phase was 28:72 (v/v). 1 M KOH was used for the reaction solution. Special grade ethanol (99.5%) was used for the cleaning solution. The flow rate of the mobile phase, reaction solution, and cleaning solution were 1.0 ml/min, 0.5 ml/min and 2.0 ml/min, respectively. The temperature of both columns was approximately 60 °C. A 50 μl aliquot of a two-fold diluted sample in mobile phase was injected. This system was constructed in accordance with the first supplement of the Japanese Pharmacopoeia (15th edition).

2.4.2. Dialysis method

Rate of PGE_1 retention in lipid particles was measured by a dialysis method. 340 μl of Lipo-PGE_1 was injected into a dialysis cassette, immersed in 85 ml of each infusion solution, and incubated with gentle agitation at 20 °C in a water bath. Each dialysis cassette was floated by using a buoy. After incubation for the indicated time the dialysis cassette was retrieved and the concentration of PGE_1 inside the cassette measured using the HPLC method described earlier. A 5 μg/ml solution of PGE_1 in normal saline was also injected into a dialysis cassette, incubated for the indicated time, and then the concentration of PGE_1 in the cassette measured as Lipo-PGE_1. The rate of PGE_1 retention was calculated in comparison with the 0 time sample, after correction for changes in volume of solution in the dialysis cassette.

3. Results and discussion

Firstly, we investigated whether the intravenous line becomes clogged by a suspention of generic Lipo-PGE_1 formulations and Hartmann's solution pH 8. A 2 ml solution of Formulation #1 (innovator) or Formulation #3 (generic) was injected into a 500 ml infusion bag of "Hartmann's solution pH 8." After mixing, an infusion set was attached to each infusion bag and the roller clamp was fully opened. In the suspension of Formulation #1, no decrease in drip rate or accumulation of aggregated substances in the infusion line was observed. By contrast, the drip rate of a suspension of Formulation #3 significantly decreased after 1 h and had completely stopped after 2 h. Additionally, a white aggregation substance was observed in the infusion line of the suspension of Formulation #3 (Fig. 1). Thus, we confirmed the previously reported phenomena.

We reasoned that an increase in the diameter of lipid particles in the infusion solution was likely to cause a decrease in the drop rate and the formation of aggregates. Therefore, after mixing of Lipo-PGE_1 with infusion solution, the time-dependent change in the mean diameter of lipid particles in the suspension was measured by dynamic light scattering (DLS) (Fig. 2). Seven different infusion solutions were used in this study as listed in Table 2. Hartmann's solution pH 8 and Ringer's lactate with sodium bicarbonate solution were included, which have been reported to cause aggregates and blockages in the infusion line. The mixing of Lipo-PGE_1 with Aminofluid® or Amicaliq® induced no obvious alteration in the size of the lipid particles over time, although the mean diameter was slightly greater compared to that observed using normal saline. The slightly greater diameter of the Lipo-PGE_1 particles in the presence of Aminofluid® or Amicaliq® is presumably caused by an accumulation of positively charged substances, such as arginine, histidine, Mg^{2+} and Zn^{2+}, around the lipid particle. In the case of Solita-T® No. 3 and Lactec® Injection, the mean diameter of the lipid particles was unchanged compared with that observed in normal saline. The mixing of Formulation #3 or Formulation #4 with the mixture of Lactec® injection and Meylon® (a 7% sodium bicarbonate injection) transiently increased the mean diameter of the lipid particles, although that of innovator formulations did not. Interestingly, there was no significant increase in the mean diameter of lipid particles in Hartmann's solution pH 8.

Next, the number of large diameter lipid particles was determined in suspensions of Lipo-PGE_1 in infusion solution using a single-particle optical sensing (SPOS) method. The number of lipid

Fig. 1. Photograph of aggregation substances in suspension with Formulation #3 and Hartmann's solution pH 8.

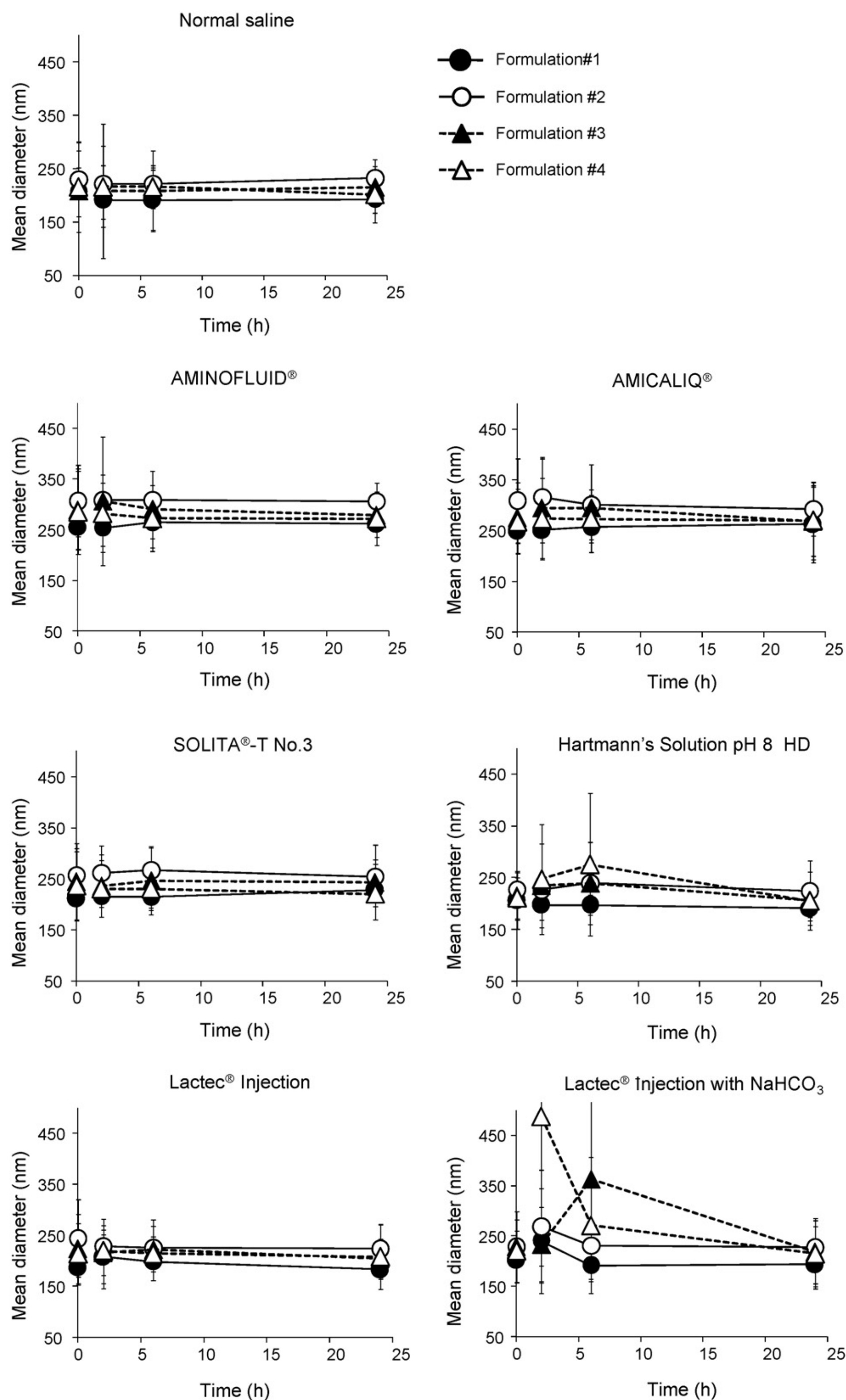

Fig. 2. Mean diameter of Lipo-PGE_1 after mixing with each infusion solution. Lipo-PGE_1 was mixed with each infusion solution and incubated at room temperature. Data represent the mean ± SD.

particles with a diameter >0.5 μm or >1 μm in an emulsion of Lipo-PGE_1 with each infusion solution is shown in Figs. 3 and 4, respectively. Intriguingly, the particle number >0.5 μm of generic formulations was larger than that of innovator formulations in all of the infusion solutions tested, while the particle number >1 μm of generic formulations was clearly smaller than that of innovator formulations in most of the infusion solutions, except Hartmann's solution pH 8 and Lactec® injection with Meylon®. Mixing generic formulations in Hartmann's solution pH 8 HD caused a significant increase in particle number especially with a diameter >1.0 μm. However, the particle number >1.0 μm of innovator formulations remained unchanged, although the particle number >0.5 μm of For-

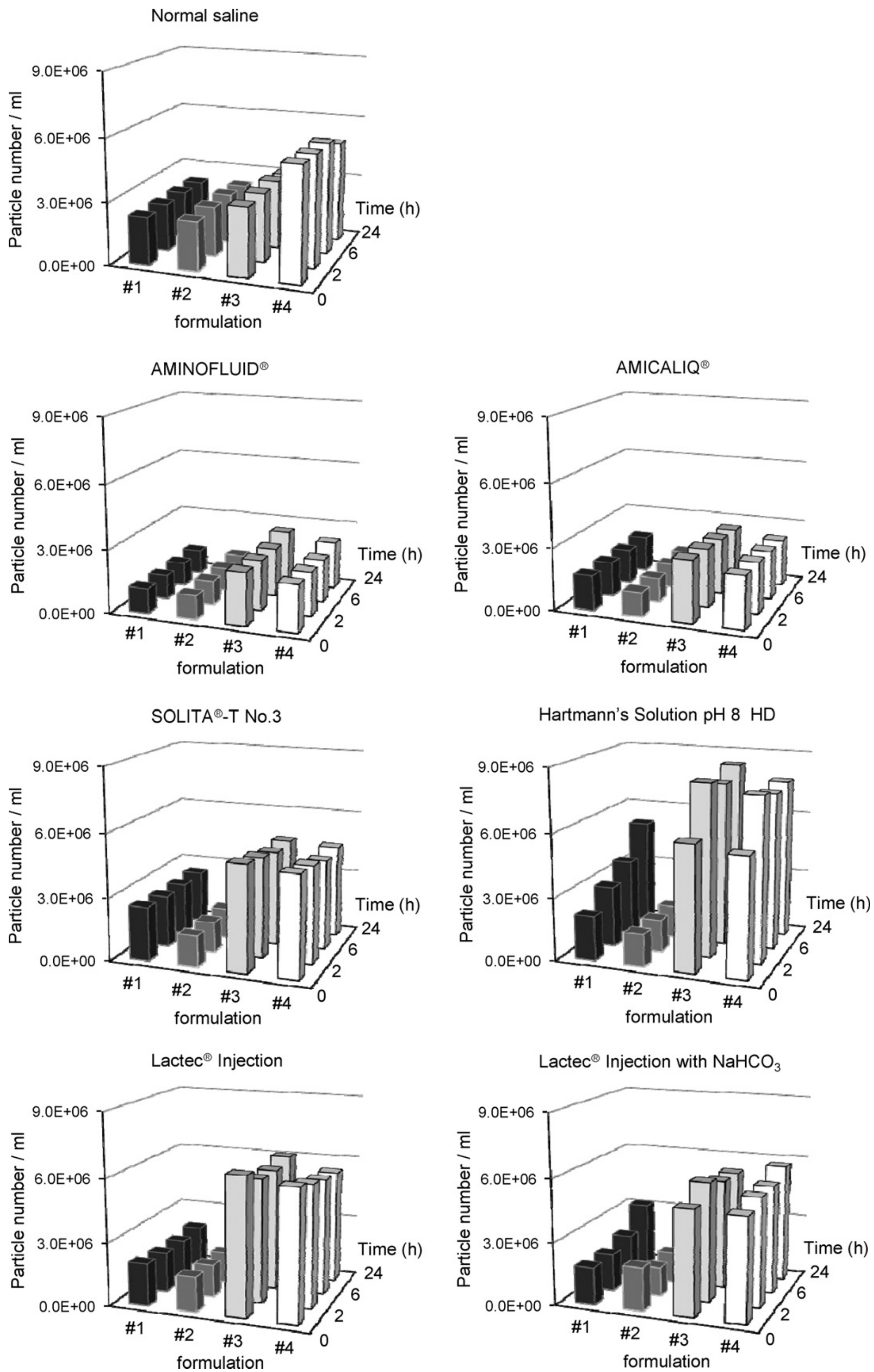

Fig. 3. Effect of each infusion solution on the total particle number of Lipo-PGE_1. The counts per milliliter per size range were normalized to the undiluted sample. Data represent the mean of two samples. Error bars were omitted by reason of the small standard deviations.

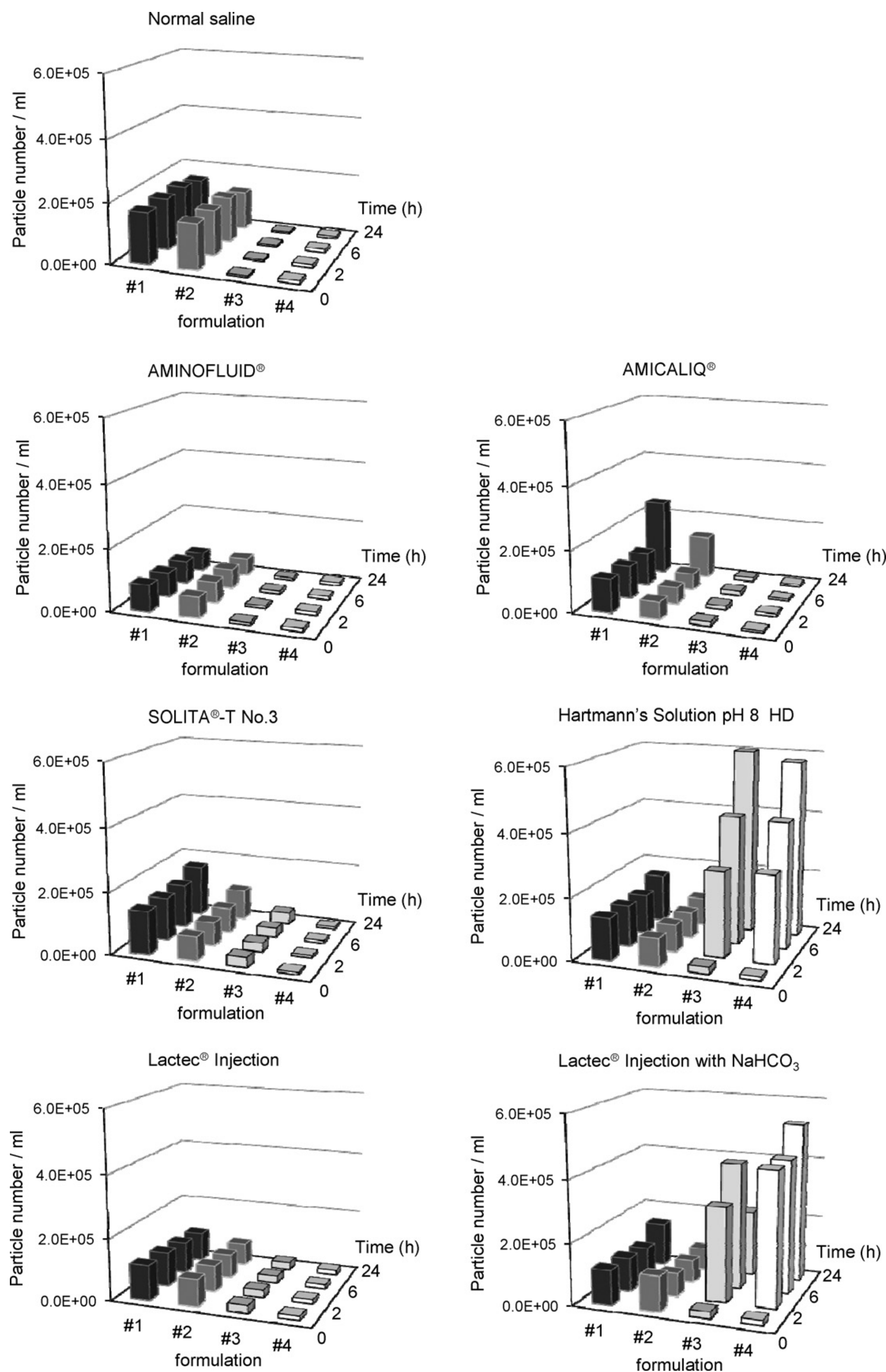

Fig. 4. Effect of each infusion solution on the large particle (diameter > 1.0 μm) number of Lipo-PGE_1.

mulation #1 gradually increased over time. Furthermore, in Lactec® injection with Meylon®, the particle number >1.0 μm of generic formulations increased markedly. Fig. 5 shows the particle distribution in an emulsion of each formulation with Hartmann's solution pH 8 or Lactec® injection with Meylon®. Among the innovator formulations, the distribution curve of Formulation #2 was unchanged, even 24 h after mixing, although that of Formulation #1 displayed an increase in the number of large particles. For the generic for-

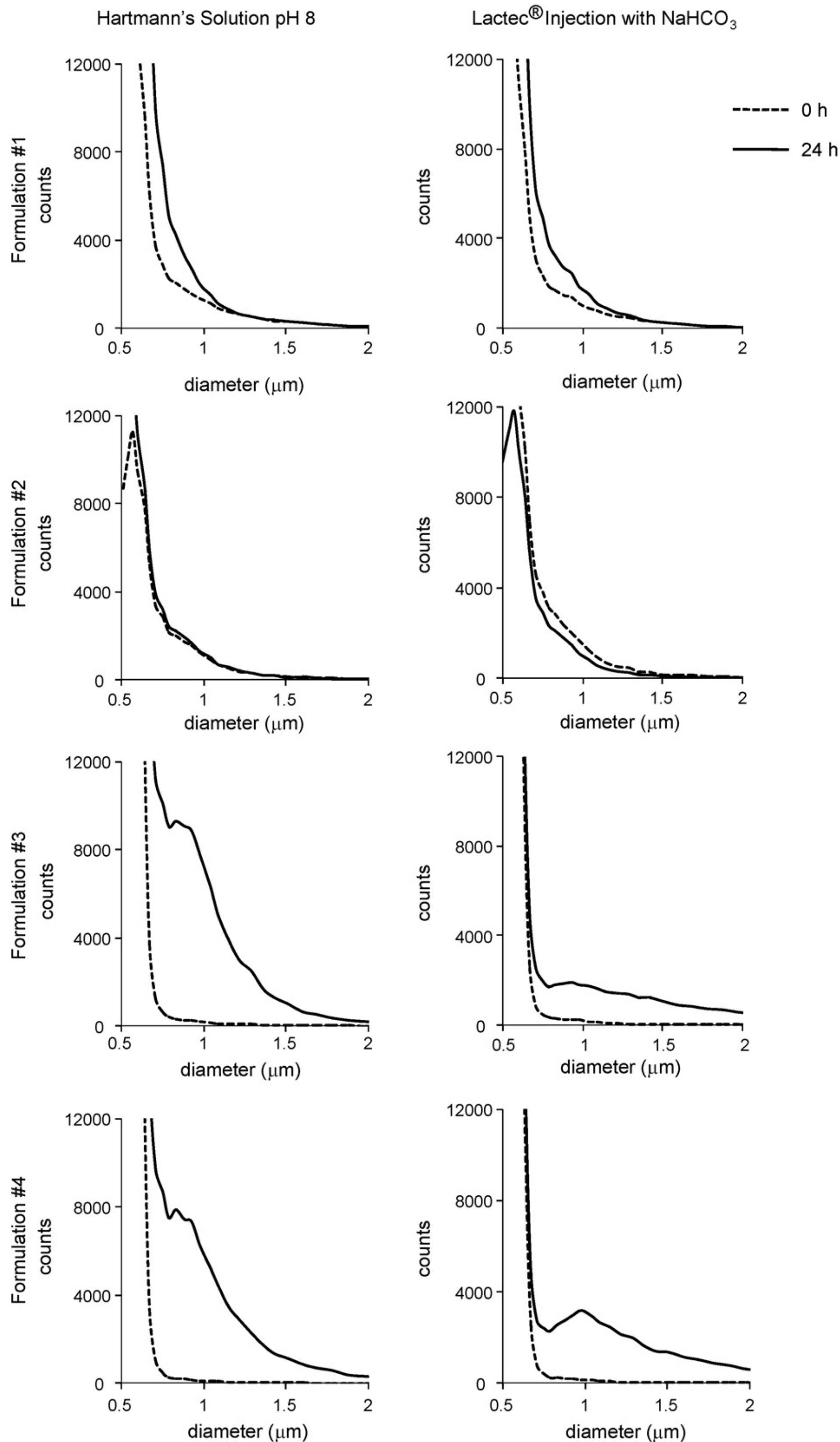

Fig. 5. distribution of Lipo-PGE_1 in Hartmann's solution pH 8 or Lactec® Injection with $NaHCO_3$. Dashed lines show 0 time, and full lines show 24 h after mixing with Lipo-PGE_1 and each infusion solution.

mulations, a significant increase of lipid particles of around 1 μm in diameter or a broad distribution of large particles was observed in Hartmann's solution pH 8 or Lactec® injection with Meylon®, respectively. These results indicate that the increase in the size of the lipid particles is related to the infusion solution used. The change in the size of the lipid particles in Amicaliq® was observed over a period of time. The particle number of innovator formulations increased 24 h after mixing, while that of generic formulations did not. In other infusion solutions there were no change in the particle number.

Table 3
Volume-weighted percentages of lipid particles with a diameter of >5 μm ($PFAT_5$) 2 h after suspension.

$Lipo\text{-}PGE_1$	Normal saline	Mean ± S.D.	
		Hartomann's solution pH 8 HD	Lactec® Injection with $NaHCO_3$
Formulation #1	0.025 ± 0.006	0.026 ± 0.002	0.009 ± 0.001
Formulation #2	0.006 ± 0.001	0.007 ± 0.001	0.007 ± 0.001
Formulation #3	0.034 ± 0.002	0.014 ± 0.004	0.056 ± 0.004
Formulation #4	0.012 ± 0.003	0.008 ± 0.001	0.012 ± 0.002

These results indicate that the substantial increase in the number of large particles is a potential factor leading to the aggregation of substances in the infusion line and slowing of the drip rate. Moreover, our studies revealed that, even using innovator formulations, the mixing of Formulation #1 with Hartmann's solution pH 8 increased the number of large particles at some level. Thus, in some cases, the SPOS method could detect changes in the number of large particles that could not be detected by the measurement of mean diameter or size distribution using DLS. Our data also indicate that the SPOS method may be a useful means of assessing the formulation stability of emulsions or the incompatibility with infusion solutions. Although the actual number of particles is given in this study, in USP ⟨729⟩, the instrument range of detection is set at 1.8–50 μm, and the volume-weighted percentage of lipid particles greater than 5 μm ($PFAT_5$) must be less than 0.05%. Thus, the $PFAT_5$ of each $Lipo\text{-}PGE_1$ formulation was calculated after mixing with normal saline, Hartmann's solution pH 8, or Lactec® injection with Meylon® (Table 3). Only the $PFAT_5$ of Formulation #3 in Lactec® injection with Meylon® was more than 0.05%, and the $PFAT_5$ of even generic formulations did not exceed 0.05%. This is because there was a significant increase in the number of lipid particles around 1 μm in diameter in the emulsion of generic formulations with Hartmann's solution pH 8. Such emulsion conditions could result in a blocked infusion line or at least some build-up of aggregation substances. Therefore, a measurement of the number of particles not only larger than 5 μm but also around 1 μm will be required depending on the particular situation. Large particles ranging from dozens to several hundred μm in diameter, which can clog the infusion line directly, were not detected in our study. There is a possibility that the partial accumulation of lipid particles around 1 μm may trigger a blockage in the infusion line. However, we could not eliminate the existence of large particles that are not detected by the SPOS method because they may be unstable in the very high flow rates used in this technique. Nevertheless, the assessment of emulsions using the SPOS method will simplify the procedure and allow the detection of a wide distribution of particle sizes.

One factor that may increase the number of large particles in the emulsion of generic $Lipo\text{-}PGE_1$ but not innovator $Lipo\text{-}PGE_1$ under alkaline conditions in the presence of calcium ions, such as Hartmann's solution pH 8 or Lactec® injection with Meylon®, is the zeta potential of the particle surfaces. As is well known, an electric charge on the particle surface brings about inter-particle repulsion, thereby preventing aggregation (Washington et al., 1989; Washington, 1990). If the electric charge of the particles in the emulsion is small, the stability of the emulsion will decrease. Thus, the zeta potential of each $Lipo\text{-}PGE_1$ formulation in water was measured (Table 4). Surprisingly, there were no significant differences between innovator and generic formulations. Indeed, the zeta potential of the generic formulations was slightly lower than that of the innovator formulations. This result indicates at least that the difference of zeta potential in normal saline may not be a factor causing the difference in stability between generic and innovator formulations under alkaline conditions in the presence of calcium ions. To clarify the effect of zeta potential on the formation of aggregation, the zeta potential value of each formulation in all infusion solutions as well as various pH solutions in the presence or absence of calcium ions should be measured as a subject of future investigation. Other factors include not only the difference in the method for manufacturing or the control of the process, but also a difference in the formulation. While the lipid particle of innovator formulations is composed of soybean oil, that of generic formulations is composed of olive oil as shown in Table 1. These two kinds of plant oil seem to have some differences in their physical characteristics because the main fatty acid of soybean or olive oil is linoleic acid or oleic acid, respectively. All additives in the generic formulations, except the oil, are exactly the same as innovator formulations. The hydrophile–lipophile balance (HLB) of emulsifier or oil as well as temperature and pH is important for the stability of the emulsion (Griffin, 1954; Becher, 1957). Thus, there is a possibility that the change of plant oil might bring about an alteration in the appropriate HLB of lecitin, and cause instability within the emulsion such as aggregation. Additionally, the HLB of lecitin is not constant like 7–13, because lecitin is a mixture of emulsifiers. Therefore, the HLB, relative proportion, or purity of lecitin might bring about the observed difference in stability. In either case, a detailed investigation will be required to reveal the cause of the increase in the number of large particles in generic formulations under alkaline conditions in the presence of calcium ions.

One report suggests that the PGE_1 retention rate of generic formulations in lipid emulsions is lower than that of the innovator formulations (Takenaga et al., 2007). $Lipo\text{-}PGE_1$ should be present at a high concentration within a lesion and maintain the concentration and activity of PGE_1 in the circulatory system. Stable retention of PGE_1 within the lipid particle is very important for exhibiting clinical effectiveness. Thus, the PGE_1 retention rate was measured in normal saline, Solita®-T No. 3, and the infusion solution in which the number of large particles increased, such as Amicaliq®, Hartmann's solution pH 8, and Lactec® injection with Meylon® (Fig. 6). The PGE_1 retention rates were measured using either the filtration, dialysis, or ultrafiltration method in previous reports (Takenaga et al., 2007; Igarashi et al., 1988; Yamaguchi et al., 1995; Teagarden et al., 1988). In the filtration method, the sample is passed through a membrane filter with a pore size of 0.1 μm connected to a disposable syringe. However, smaller particles may pass through the filter unit causing the data to fluctuate depending on the precise force applied to the syringe. In this study, the PGE_1 retention rate was measured by the dialysis method. Compared to a solution of PGE_1, each formulation clearly retained PGE_1 in lipid particles in normal saline, Solita®-

Table 4
Zeta potential of $Lipo\text{-}PGE_1$.

Peak position	Formulation #1 (innovator)	Formulation # 2 (innovator)	Formulation # 3 (generic)	Formulation # 4 (generic)
Mean (mV)	−23.1	−24.5	−29.5	−28.3
Width (mV)	6.0	7.1	6.1	4.9

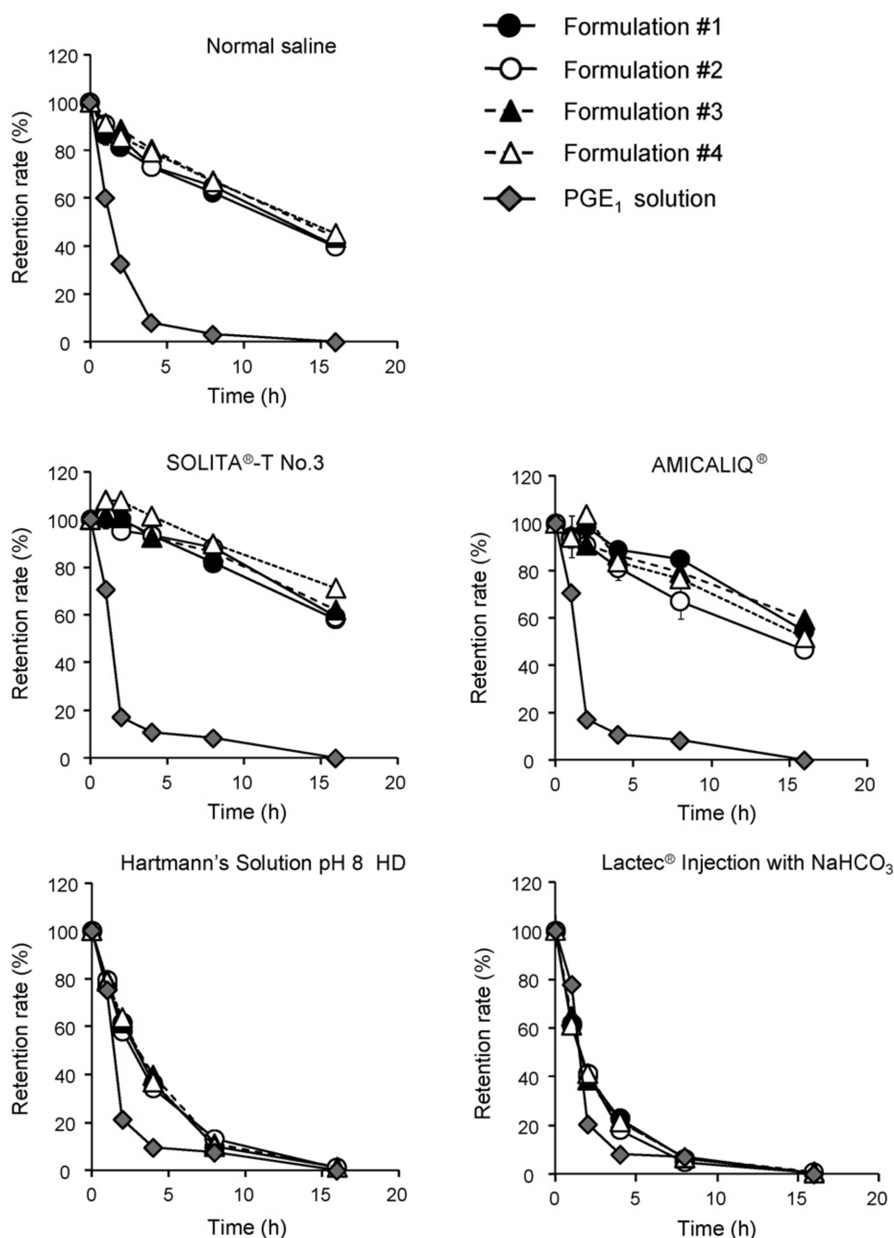

Fig. 6. Retention rate of PGE_1 in lipid particles in each infusion solution. Retention rate of PGE_1 in lipid particles of Lipo-PGE_1 was measured by the dialysis method at 20 °C. Plots show mean ± SD of percentages for three individual experiments. Solution PGE_1 (closed diamond) was used for a control of Lipo-PGE_1.

T No. 3 and Amicaliq® (Fig. 6). Moreover, there were no significant differences between formulations in these three infusion solutions. PGE_1 was rapidly released from lipid particles in Hartmann's solution pH 8, and Lactec® injection with Meylon®. Indeed, almost no PGE_1 remained in the lipid particles after 8 h. These results correlate with previous reports which indicated that the PGE_1 retention rate was influenced by the pH of buffered solution (Yamaguchi et al., 1995). This is because in alkaline condition PGE_1 ($pK_a = 4.89$) is anionic form and readily released from lipid particled while in acidic condition PGE1 is nutral form and mainly distributed to the oil/water interface (Teagarden et al., 1988). Even in these two infusion solutions, in which an increase in the number of large particles was observed, differences in the PGE_1 retention rate between innovator and generic formulations were not detected. Therefore, our results indicate that the PGE_1 retention rate of generic formulations is no lower than that of the innovator formulations.

Our study shows that the marked increase of large diameter (>1 μm) particles is a likely reason for the slowing of the drip rate and aggregation of substances in an infusion line of generic formulations under alkaline conditions in the presence of calcium ions. Moreover, our study indicates that a measurement of the number of large particles is very effective in assessing the stability of an emulsion. We also show that the mixing of Lipo-PGE_1 with Hartmann's solution pH 8 or Lactec® injection with Meylon® significantly decreased the retention rate of PGE_1 in lipid particles compared to normal saline. This decreased retention rate of PGE_1 is undesirable if Lipo-PGE_1 is to exert its full clinical effectiveness. Actually, the mixing of Lipo-PGE_1 with medicines other than infusion solution is restricted according to the package insert. However, the application of Hartmann's solution pH 8 is unrestricted because it is an infusion solution. Care must be taken over the choice of infusion solution due to the increase in the number of large particles generated using the generic formulations. Furthermore, it is preferable to administer Lipo-PGE_1 at as low a dilution as possible. In conclusion, except under alkaline conditions in the presence of calcium ions, there is no difference in mixing any infusion solu-

tion with Lipo-PGE_1. Specifically, there is no differences between generic formulations and innovator formulations in terms of PGE_1 retention rate in lipid particles. Thus the clinical effect of generic formulations will not differ greatly from that of innovator formulations.

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