Pharmaceutical Characterization of Commercially Available Intravenous Fat Emulsions: Estimation of Average Particle Size, Size Distribution and Surface Potential Using Photon Correlation Spectroscopy[1)]

Hiroaki KOMATSU,* Aya KITAJIMA, and Satoshi OKADA

Division of Drugs, Osaka Branch, National Institute of Health Sciences, 1–1–43, Hoenzaka, Chuo-ku, Osaka 540, Japan. Received February 8, 1995; accepted April 13, 1995

Particle profiles such as the average diameter, size distribution and dispersion, as well as the zeta potential, of commercially available intravenous fat emulsions of high-calorie nutrient fluids (6 products) and drug carriers (4 products) were examined using photon correlation spectroscopy (dynamic light scattering). Wide variations were observed in number-weighted (*dn*), weight-weighted (*dw*) and z-average diameters, and *dw*/*dn* ratios as a measure of polydispersity. Average size is not sufficient for the pharmaceutical characterization of particles and the determination of size distribution or *dw*/*dn* value is essential for more precise information. Although measuring the zeta potential of fat emulsions is of considerable value in estimating their stability on long-term storage, a medium which accurately reflects the environment of the droplets in the system of interest should be chosen when diluting the emulsion.

Key words fat emulsion; average particle size; zeta potential; surface potential; stability; lipid emulsion

Numerous methods for using small lipid-particles as fat emulsions and liposomes for the transport of lipophilic and/or hydrophilic drugs have been developed and a number of them have been put to practical use.[2,3)] Recently, the novel idea that they can also be used as drug delivery systems has been proposed with advanced functions such as targeting drugs to specific tissues or organs.[2,3)] Such special functions are dependent on surface modification, size control, the presence of a surface charge and a combination of these, and the development of emulsions for practical use has made steady progress.[3)] In the near future, commercial products using these particle dispersions will increase in number, and, therefore, universal estimation methods for their pharmaceutical characterization are urgently required.

Photon correlation spectroscopy (PCS) is a laser-light scattering technique which uses fluctuations in scattered light intensity to measure the velocity of Brownian diffusion of small particles and, hence, reflects their diameters.[4)] As it is quite sensitive to particles whose diameter ranges from 3 nm to 3 μm, it can detect the majority of small lipid particles. It has also been suggested that the ability to measure zeta potential is of considerable value in evaluating the stability of any colloidal system.[4–6)] The direct microscopic observation which was first used has been largely replaced by light scattering methods, in which the particle velocity during electrophoresis is obtained from the Doppler shift of light scattered from the moving particles.[7)]

In the present study, intravenous high-calorie nutrient fluids and drug carriers of alprostadil (prostaglandin E_1), dexamethazone palmitate and flurbiprofen axetil were selected as commercially available intravenous fat emulsions, based on soya oil in water stabilized by lecithin obtained from eggs. Particle profiles such as the average diameter, size distribution and polydispersity were estimated using the PCS technique and these were compared for different preparations. Their zeta potentials were also determined as a measure of surface potential using the PCS technique combined with electrophoresis.

Experimental

Materials Intralipid®, Intrafat® and Intralipos® were purchased from Ohtsuka Pharmaceutical, Ltd., Tokushima, Japan (coming originally from Kabi Pharmacia AB, Uppsala, Sweden), Nihon Pharmaceutical, Co., Ltd., Tokyo, Japan and Green Cross, Co., Ltd., Osaka, Japan, respectively. Limetason®, Lipfen® and Liple® were obtained Green Cross, Ltd., Osaka, Japan. Palux® was obtained from Taisho Pharmaceutical, Co., Ltd., Tokyo, Japan. Data on their lipid concentration, volume, lot or product number, components and contents are summarized in Tables 1 and 2. All other agents were of analytical grade.

Measurements PCS: In order to estimate average size, size distribution and zeta potential, PCS of fat emulsions was carried out using a Zetasizer 4 photon-correlation spectrometer from Malvern Instruments (Worcs., U.K.) at 25 °C. For the particle size measurements, all samples were measured within two months of receipt. After moderate shaking, samples were diluted with buffer solution (adjusted to pH 7.0 with HCl solution) containing 0.15 M NaCl and 1 mM Tris. Particle size was measured using a ZET 5104 cell in parallel beam mode, and light scattering was observed at 90°. Further details are given in our previous papers.[8,9)]

In the case of zeta potential measurements, all samples were measured within 6 months of receipt. Samples were diluted with distilled water, buffer solution or the continuous phase obtained from centrifugion of each fat emulsion ($\times 100000\,g$, for 1 h, at 15 °C). Zeta potentials were measured using a ZET 5103 small capillary cell in cross beam mode,

Table 1. Product Names, Volumes and Product/Lot Numbers of Commercially Available Fat Emulsions

Product names[a)]	Volume (ml)	Product/Lot No.
Intralipid 20%	100	54301-51
Intralipid 20%	250	54111-51
Intrafat inj.	250	S129
Intrafat inj. 20%	500	S113
Intralipos 10%	250	11918HI
Intralipos 20%	250	20916HI
Limethason	1	113HI
Lipfen inj.	5	022HI
Liple	1	368HI
Palux inj.	2	196K1

a) The product names are registered trademarks belonging to each company.

* To whom correspondence should be addressed.

Table 2. Compositions and Contents of Commercial Fat Emulsions

Product names	Effective components		Additives	
	Name	Concentration	Name	Concentration (mg/ml)
Intralipid 20% (54301-51)	Purified soybean oil	200 mg/ml	Purified egg lecithin	12
			Conc. glycerin	22.5
			NaOH	q.s.[a]
Intralipid 20% (54111-51)		200 mg/ml	Purified egg lecithin	12
			Conc. glycerin	22.5
			NaOH	q.s.[a]
Intrafat inj.		100 mg/ml	Purified egg lecithin	6
			Conc. glycerin	22.5
			NaOH	q.s.[a]
Intrafat inj. 20%		200 mg/ml	Purified egg lecithin	12
			Conc. glycerin	22.5
			NaOH	q.s.[a]
Intralipos 10%		100 mg/ml	Purified egg lecithin	6
			Conc. glycerin	22.5
			NaOH	q.s.[a]
Intralipos 20%		200 mg/ml	Purified egg lecithin	12
			Conc. glycerin	22.5
			NaOH	q.s.[a]
Limethason	Dexamethazone palmitate	4.0 mg/ml	Purified egg lecithin	12
			Purified soybean oil	100
			Conc. glycerin	22.1
Lipfen inj.	Flurbiprofen axetil	10 mg/ml	Purified egg lecithin	18
			Purified soybean oil	100
			Oleic acid	2.4
			Conc. Glycerin	22.1
Liple	Alprostadil	5 μg/ml		
Palux inj.		5 μg/ml		

a) Quantum sufficient.

and 100 V was applied during the electrophoresis (the current was below 0.5 mA. For the samples diluted with the continuous phase obtained from centrifugation of the original sample solution, the content of glycerin in the medium was taken into consideration when conducting the analysis.

Results and Discussion

Particle Size Figure 1 shows typical size distributions (weight-weighted diameter distributions) for a variety of fat emulsions. Almost all the commercial fat emulsions gave mono-dispersed distributions except for Intralipos (54111-51), and the distribution ranges were relatively narrow. Intralipos (54111-51), in contrast, showed two distribution peaks and there was a wide distribution of diameters. This may have been due to from secondary coalescence of emulsion particles.

Table 3 presents a comparison of size parameters, number-weighted (*dn*), weight-weighted (*dw*) and z-average (*dz*) diameters and *dw*/*dn* of various fat emulsions. Clear differences were seen, with size averages ranging from 180 nm to 344 nm in *dz*. In particular, fat emulsion particles of drug carriers were smaller than those of high-calorie nutrient fluids. This may be due to smaller ratios of soya oil to egg yolk lecithin in the drug carriers (oil/emulsifier = 6—8 by weight) than in the high-calorie nutrient fluids (oil/emulsifier = 17 by weight), as shown in Table 2. Handa *et al.*[13] and we[9] have demonstrated that the size of fat emulsions is clearly dependent on oil to emulsifier ratios, and that a relative increment in an emulsifier can lead to smaller fat emulsions.

Table 3 also contains *dw*/*dn* values as measures of polydispersity and the nearer the value is to 1 the narrower is the distribution. The *dw*/*dn* values were below 1.1, and almost all the commercial fat emulsions had very narrow size distributions.

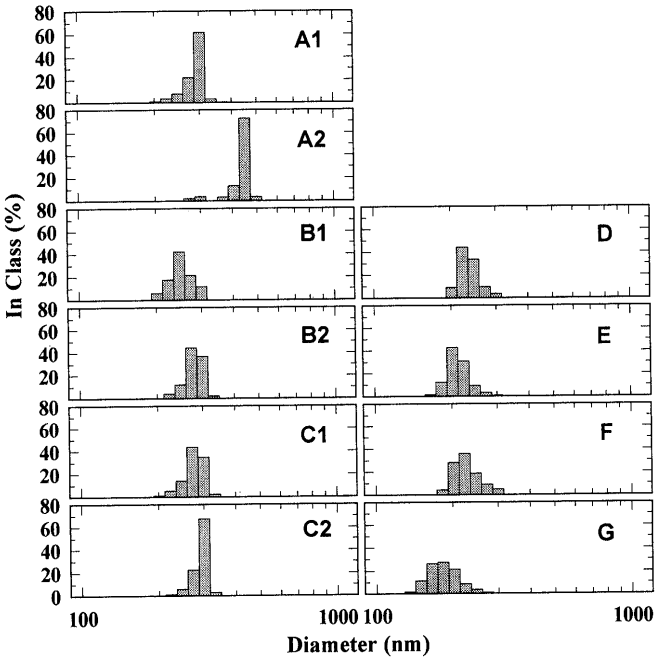

Fig. 1. Typical Weight-Weighted Particle-Size Distributions of Commercially Available Fat Emulsions

A1, intralipid 20% (54301-51); A2, intralipid 20% (54111-51); B1, intrafat inj.; B2, intrafat inj. 20%; C1, intralipos 10%; C2, intralipos 20%; D, limethason, E, lipfen inj.; F, liple; and G, palux inj.

The wide variation observed in the average sizes of *dn*, *dw* and *dz*, the size distributions suggest that commercially available fat emulsions form a relatively heterogeneous group.

Emulsion droplets exceeding 6 μm in diameter are

Table 3. Typical Size Parameters of Various Fat Emulsions

Product names	Average size (nm)			
	dn	*dw*	*dz* Average ± S.D. ($n=30$)	*dw*/*dn*
Intralipid 20% (54301-51)	279.6	287.9	309.5 ± 5.3	1.03
Intralipid 20% (54111-51)	285.1[a] 436.1[a]	290.3[a] 441.3[a]	344.0 ± 12.9	—
Intrafat inj.	242.6	251	224.3 ± 4.9	1.03
Intrafat inj. 20%	272.3	278.7	255.6 ± 5.4	1.02
Intralipos 10%	269.3	276.6	257.8 ± 6.1	1.03
Intralipos 20%	286.6	291.2	273.7 ± 6.1	1.02
Limethason	230.5	236.1	222.6 ± 5.7	1.02
Lipfen inj.	206.1	212.2	202.9 ± 3.6	1.03
Liple	218.9	227.5	199.3 ± 3.6	1.04
Palux inj.	174.5	183.9	179.6 ± 2.6	1.05

a) Two peaks were observed in the size distribution.

Table 4. Zeta Potential and pH of Commercial Fat Emulsions

Product names	Average ± standard deviation (number of determinations, *n*)			
	Zeta potential (mV)			pH of original solution ($n=3$)
	In dist. water (*n*)	In the continuous phase of the emulsion ($n=5$)	In buffer (0.15 M NaCl, 1 mM Tris, pH 7) (*n*)	
Intralipid 20% (54301-51)	−90.8 ± 2.2 (20)	−33.0 ± 0.9	−4.2 ± 2.2 (20)	7.84 ± 0.40
Intralipid 20% (54111-51)	−83.6 ± 0.7 (15)	−24.1 ± 1.9	−3.9 ± 0.7 (26)	7.65 ± 0.13
Intrafat inj.	−83.9 ± 1.5 (15)	−61.8 ± 1.6	−5.7 ± 0.5 (10)	7.51 ± 0.03
Intrafat inj. 20%	−59.9 ± 0.9 (15)	−69.7 ± 1.1	−6.0 ± 0.9 (35)	7.43 ± 0.11
Intralipos 10%	−89.7 ± 2.0 (30)	−45.5 ± 1.5	−4.5 ± 0.5 (25)	7.35 ± 0.07
Intralipos 20%	−69.2 ± 1.6 (15)	−48.4 ± 0.8	−4.4 ± 1.0 (15)	7.35 ± 0.05
Limethason	—	−38.2 ± 1.1	−27.4 ± 2.2 (30)	6.90 ± 0.14
Lipfen inj.	—	−31.2 ± 1.6	−18.3 ± 1.0 (26)	5.72 ± 0.01
Liple	—	−32.3 ± 0.9	−18.2 ± 1.6 (37)	5.66 ± 0.10
Palux inj.	—	−15.1 ± 0.8	−11.9 ± 1.2 (18)	5.63 ± 0.05

known to cause adverse reactions, particularly emboli in the lungs.[14] Therefore, parameters reflecting the size distribution range are very important in practice. Recently, the United States Pharmacopeia (USP) proposed in rapid succession first and second drafts for the globule size distribution in intravenous emulsions.[15] Here, the PCS technique was adopted as one method of size estimation but size parameters, for precisely assessing the character of emulsions have not yet been chosen. For example, mean diameter data such as *dn*, *dw* and *dz* are on the whole very simple parameters for estimating particle size. However, they involve no information about the range of the size distribution. On the other hand, a dispersion parameter, such as the *dw*/*dn* value, is more suitable, particularly when there is a broad size distribution. In the USP draft, a chi-squared value, which describes the goodness of fit of the analysis, with the particle size distribution having an approximately log normal or Gaussian shape, has also been adopted as one of the characteristics for assessing size distribution.[15]

Thus, average size is not in itself sufficient for the pharmaceutical characterization of particle size.

Zeta Potential Table 4 shows the zeta-potentials for various fat emulsions diluted in an original medium (the continuous phase solution obtained from the centrifugation of each original emulsion). A medium which accurately reflects the environment of the droplet in the system of interest should be chosen for the dilution procedure, as discussed in the next section.

Although the estimated zeta potentials varied greatly, they were all negative. The charge on the droplet arises from the egg lecithin covering the particle surface. The lecithin used as an emulsifier is a heterogeneous mixture of phospholipids; that used as an intravenous emulsifier is highly purified but still contains a wide variety of materials.[4] The majority of the lipids (80—90%) consists of phosphatidylcholine and phosphatidylethanolamine, uncharged at physiological pH. It also contains smaller (2—5%) quantities of acidic lipids, largely phosphatidylserine and phosphatidylglycerol.[4] In the case of Liple and Palux Inj., especially, oleic acid is also present as a lipid. These lipids are negatively ionized around pH 7 and so confer a negative surface-charge on the emulsion droplets.

The zeta potentials of high-calorie nutrient fluids ranged from −25 to −70 mV when samples were diluted in the original solution medium. On the other hand, those of fat emulsions for drug carriers ranged from −15 to −38 mV. In Liple and Palux Inj., a significant difference in zeta potential was found in spite of them having the same components and contents. The lecithin in these cases is a mixture of phospholipids whose exact composition depends on the source, usually eggs, and to a smaller extent on environmental factors at work during its *in vivo* synthesis.[6] It has been suggested that the production of free acid from lecithin and triglycerides may account for changes in the surface charge of fat emulsions with age.[16] Therefore, the differences in the observed zeta potentials may be ascribed to differences in the origin of the egg yolk lecithin and/or the period after preparation (ageing effects).

Problems with Zeta Potential Measurements Using PCS and Their Solution Lipid emulsions are very turbid due to the large difference in reflective index between medium and core oil and they must be diluted using appropriate solutions before light scattering measurements can be performed. The zeta potential of fat emulsions diluted with distilled water or physiological saline also shown in Table 4 bear no resemblance to those of the emulsions in their original solutions.

The concentration of dominant ions was reduced by some orders of magnitude. It is not appropriate to dilute emulsions with distilled water or physiological saline since the zeta potential then measured is that of the emulsion in water and does not reflect the potential stabilizing the emulsion. This suggests that a medium which accurately reflects the environment of the droplet in the system of interest should be chosen. In the case of model studies in sample electrolyte solutions, this presents little difficulty; however, it is much more difficult to measure the zeta potential of a particle in a fat emulsion. In order to obtain a relevant zeta potential, it is

necessary to maintain the continuous phase composition on dilution. There are two approaches to this problem; if the composition of the continuous phase is known, it can be prepared without any emulsion component and used as the diluent. In the present situation the continuous phase composition was uncertain and this is very common. In this case, the usual answer is to centrifuge the dispersion to obatin a sample of the continuous phase for dilution, as demonstrated by Washington *et al.*[4)]

In conclusion, i) wide variations were observed between commercial preparations in terms of the average sizes of *dn*, *dw* and *dz*, the *dw*/*dn* ratios, and the size distributions; ii) not only average sizes but also size distributions and polydispersities are essential to precise pharmaceutical preparations; and iii) although measuring the surface potential of fat emulsions is of considerable value in estimating their stability, a medium which accurately reflects the environment of the droplet in the system of interest should be chosen for diluting the emulsion.

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