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Physicochemical assessments of parenteral lipid emulsions: light obscuration versus laser diffraction

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Abstract

The United States Pharmacopeia (USP) has proposed a new Chapter ⟨729⟩ entitled 'Globule Size Distribution in Intravenous Emulsions' that is intended to identify methods for analyzing the stability of lipid emulsions. We studied the differences between particle-sizing instruments when analyzing the physicochemical stability of a parenteral nutrition mixture compounded with intravenous lipid emulsion, known as an all-in-one mixture. As the growth of lipid droplets, i.e. coalescence, signals an irreversible change in emulsion stability, we focused our investigation on the large diameter tail (> 5 μm) of the globule size distribution. Of the four proposed methods, droplet size was studied over a range of mixture stabilities using a low osmolality parenteral nutrition formula employing both light scattering and light obscuration techniques. In addition, the same mixtures were also freshly prepared, and then spiked with a known amount of 5 μm latex spheres. The response obtained from the light obscuration technique was linear and detected both unstable and latex-spiked mixtures in every case for droplets or particles > 5 μm. The results of the laser diffraction method were non-linear and overestimated, was less sensitive or missed entirely, globules or particles in the large diameter tail of the dispersion. The results demonstrate that light obscuration is superior to laser diffraction in identifying unstable intravenous fat emulsions.

1. Introduction

For the determination of particulate matter in parenteral products, Chapter ⟨788⟩ of the United States Pharmacopeia (USP) specifies a two stage test in which light obscuration particle counting is

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the initial test applied to assess conformance with particle count limits at ≥ 10 and ≥ 25 μm (The United States Pharmacopeia, 2000). These limits are specified in order to ensure that randomly sourced particles, inevitably appearing in the production process, do not exceed certain levels. These limits also serve to ensure that monotypic particles present due to process or product defects are not allowed to exceed specified levels. Particle numbers below the USP limits are without detectable physiologic consequence. While collateral circulation through the capillaries and arterioles of the lung readily compensate for any particle exposure due to administration of parenteral solutions complying with the ⟨788⟩ requirement, loading of the pulmonary vasculature due to injectables which are particulate-laden in nature (e.g. emulsion containing admixtures) remains a concern. Pulmonary embolism may emanate from the smallest of blood vessels, such as capillaries, with diameters between 4 and 9 μm, or in relatively larger vessels such as arterioles, that have internal diameters slightly smaller than 20 μm (Guyton, 1991). Thus, the limits of the USP Particle Count Tests outlined in Chapter ⟨788⟩ are consistent with these physiological dimensions. This Chapter is specifically intended for aqueous parenteral solutions.

For particle size assessments of other parenteral products, such as intravenous emulsions, the USP recently previewed a new Chapter ⟨728⟩, entitled 'Globule Size Distribution in Intravenous Emulsions' (Globule Size Distribution in Intravenous Emulsions, 1991). The physicochemical stability of intravenous lipid emulsions (IVLEs) has been characterized by various methods of analysis. When the coalescence of fat globules is used as an endpoint of emulsion stability, assessments of the changes in the globule size distribution (GSD) have been made using a variety of different techniques. These include: optical microscopy; light obscuration (LO), also known as light extinction (LE); electrical-sensing zone (ESZ); photon correlation spectroscopy (PCS), also known as dynamic light scattering (DLS); and laser diffraction (LD), also known as Fraunhofer diffraction (FD). In 1994, the USP published the first 'in-process' revision of the aforementioned newly proposed Chapter, in which only two methods of assessing lipid emulsion stability were presented (Globule Size Distribution in Intravenous Emulsions, 1994). Specifically, these included PCS, used to identify the mean droplet size, and ESZ, used to provide particle count/size information in the upper size range of the GSD. Thus, two regions of the overall GSD would be evaluated in the proposed Chapter ⟨728⟩. In subsequent revisions, now referred to as Chapter ⟨729⟩, the LD and LO methods have been added (Globule Size Distribution in Intravenous Emulsions, 1995, 1998). In addition, Chapter ⟨729⟩ now states that 'it might not be appropriate to provide a single reference method against which all other methods are to be validated' (Globule Size Distribution in Intravenous Emulsions, 1998). Thus, in its present form, the new Chapter implies that the application of any of the four proposed methods that assesses both mean droplet size and the upper size range of the GSD may be used to satisfactorily meet compendial requirements, and thus to validate the stability of a commercial lipid emulsion product. Furthermore, no size limits for effective use of the methods are suggested in the most current version of the proposed Chapter.

There are several combinations of the methods of tests currently named in the latest revision of ⟨729⟩ that may be satisfactory for providing the necessary assessment of the mean and large diameter tail of the GSD. In fact, light microscopy has been widely used for large globules (> 5 μm) both in the U.S. and Europe for many years. In a specific research or quality control laboratory, specific combinations such as LD and microscopy or PCS and LO may have desirable advantages. The key concern here is that the methodologies used must be appropriately validated according to the USP ⟨1225⟩ criteria (e.g. reproducibility, robustness, precision, etc.) to provide an acceptable result.

The physicochemical stability of IVLEs is accomplished using a mixture of phospholipids from egg lecithin, classified as an amphoteric emulsifying agent that preferentially adsorbs to the oil droplet at the oil–water interface. The non-polar, fatty acid tails orient toward the oil phase, whereas the ionized polar phosphate groups extend into the aqueous phase. The resultant net

negative charge imparted to the oil droplet surfaces establishes mutual repulsion between neighboring droplets, thus stabilizing the emulsion. This electrostatic surface charge gives rise to a zeta potential that ideally lies in the range of -30 to -50 mV, thereby conferring stability to the droplets. All currently available IVLEs used in parenteral nutrition support have a standard pH range between 6.0 and 9.0, that maintains the surface charge and conforms with the USP proposed monograph, entitled 'Intravenous Fat Emulsion' (Intravenous Fat Emulsion, 1998). Use of lower pH values rapidly reduces the zeta potential, thus compromising emulsion stability. Moreover, an increase in the concentration of other positively-charged, adsorbing cations, such as magnesium and calcium, invariably added during the extemporaneous preparation of all-in-one mixtures, results in their competitive adsorption to the negatively charged lipid droplets. This mechanism also reduces the net surface charge, which, therefore, further reduces emulsion stability. In either event, the adsorption of cations to the negatively charged oil droplets induces flocculation, or agglomeration, ultimately leading to the coalescence of individual lipid droplets into oversized, enlarged fat globules, thereby increasing the potential danger of the intravenous infusion. Finally, the addition of electrolytes to the lipid emulsion admixture, acts in a more general way to destabilize the colloidal dispersion of oil droplets. This is the mechanism whereby the electrostatic field emanating from a negatively-charged oil droplet, giving rise to a repulsive force on a neighboring droplet, is partially 'screened.' This effect permits Van der Waal's attractive forces between the droplets to gain influence, thus helping to promote agglomeration, as described by the DLVO theory of colloidal stability (Deryaguin, 1940; Verwey and Overbeek, 1948).

The vast majority of lipid droplets in commercial IVLEs are smaller than 0.5 μm. However, fat globules larger than 1 μ are present to varying degrees in all commercial emulsions — an expected consequence of a typical homogenization process. For example, Table 1 depicts the mean droplet size (MDS, obtained by PCS) and volume-weighted percent of fat globules (PFAT, obtained by LO) in the upper size range of the GSD (>5 μm), recently measured in our laboratories

Table 1
Mean droplet size (MDS)[a] and volume-weighted percent of fat (PFAT >5 μm)[b] in the upper size range of commercial IVLEs[c]

Product	Lot Number	MDS (nm)	PFAT% (>5 μm)
ClinOleic™ 20%	9801376	276	0.001
Critilip™ 20%	KV1249B	330	0.013
Intralipid™ 10%	12202–51	286	0.009
Intralipid™ 20%	10776–71	340	0.009
Intralipid™ 30%	16115–51	420	0.007
Lipofundin™-N 10%	8085A83	272	0.001
Lipofundin™ N 20%	8082A84	332	0.005
Lipofundin™ MCT 10%	8042A81	266	0.008
Lipofundin™ MCT20%	8075A81	287	0.009
Lipoplus™ 20%	9235A32	263	0.008
Liposyn II™ 20%	47–412-DE	278	0.007
Liposyn III™ 10%	45–351-DE	263	0.013
Liposyn III™ 20%	43–440-DE	307	0.005
Liposyn III™ 30%	41–395-DE	301	0.029
Lipovenous MCT™ 20%	KK1569	275	0.004
Structolipid™ 20%	18417–51	276	0.009
Mean Value $\pm$ S.D.		298 ± 40	0.009 ± 0.006

[a] Nicomp 370 Submicron particle sizer, particle sizing systems, Santa Barbara, CA, USA
[b] Appendix A, '1'
[c] Duplicate analyses

for a variety of IVLEs (10–30%) currently available. Creaming is the initial phase of emulsion destabilization where the electrical charge on the emulsion droplets is reduced causing them to migrate to the surface of the emulsion and alter its homogeneity. In accordance with Stoke's Law, the upward migration of oil droplets occurs as a result of the lower density of the dispersed oil phase, in relation to the continuous water phase of the emulsion. This produces a state of aggregation or flocculation of droplets into clusters. These early processes of emulsion instability are generally reversible. Once these clusters fuse together forming enlarged fat globules, i.e. coalescence, this event signals an irreversible and adverse change in the physicochemical stability of emulsions (Swarbrick, 1995). Ultimately, as the dispersion becomes less stable, the coalescence of fat globules progresses continuously until visually-obvious phase separation occurs. The process of coalescence is highly variable and strongly related to the composition of the final emulsion mixture. Under extreme-use conditions, such as those found with the extemporaneous preparation of parenteral nutrition admixtures containing IVLEs in 'all-in-one' mixtures, the emulsions are expected to be stable for at least 24 h, in accordance with clinically relevant continuous infusion times.

We have earlier attempted to quantify the growth of lipid droplets in the large-diameter tail (diameters >1.75 μm) of the GSD of all-in-one mixtures over 30 h using light obscuration (LO). We demonstrated that when the volume-weighted percentage of fat at a pre-selected threshold (PFAT) of 5 μm, exceeded 0.4% of the total lipids present, phase separation was evident to the naked eye (Driscoll et al., 1995). Thus, this concentration has been suggested as an objective threshold for determining the pharmaceutical stability of these formulations, which has been corroborated in subsequent studies (Driscoll et al., 1996, 1999, 2000a,b). This value also represents a significant point of departure in the overall GSD that may have physiological implications, such as fat embolism, if inadvertently infused in patients. As expected, the percentage of fat contained in the upper portion of the GSD in commercial emulsions is well below this value, as shown in Table 1. The proposed USP Chapter ⟨729⟩ recognizes the clinical importance of coalesced lipid droplets. Therefore, it states: 'The size of fat globules is critical since, due to mechanical filtration, larger size globules can be trapped in the capillaries of the lungs' (Globule Size Distribution in Intravenous Emulsions, 1998). Thus, the ability of a globule sizing method to identify particles in this region of the large-diameter GSD tail (i.e. diameters >5 μm) of IVLEs is a critical feature of a compendial method of analysis in order to meet pharmacopeial requirements. Quantification of the >5 μm fat globules is also necessary to identify the toxicological dose of unstable IVLEs (Driscoll, 1997).

Of the four instrumental methods proposed in Chapter ⟨729⟩, we decided to evaluate the sensitivity and accuracy of LO and LD for detection of enlarged fat globules. Based on a single-particle optical sensing (SPOS) technique, the LO method provides information about the enlarged fat globules in the upper size range of the droplet distribution. The LO instrument utilized is able to count and size individually lipid droplets larger than about 1.3 μm. The laser diffraction (LD) method provides approximate GSD information over a wide dynamic size range — from as small as 0.05 μm, to as large as 3500 μm, depending on the instrument utilized. We decided not to evaluate the PCS method, as its accuracy is mainly confined to the mean droplet diameter. While this is an important quantity in relation to the quality of the dispersion, it is a less sensitive indicator of emulsion stability. We also excluded the electrical-sensing zone (ESZ) method from our evaluation. The use of a supporting electrolyte (i.e. NaCl, Intravenous Fat Emulsion, 1998) requires more care in the validation and application of this method, and the quality of the data is often related to the experience of the individual operator. Moreover, the requirement for electrolyte by this method may induce adverse physicochemical changes in the GSD of phospholipid-stabilized emulsions. The consequences that may potentially result from the non-specific adsorption of this added electrolyte is independent of, and in addition to, any destabilization posed by the original composition of the commercial formulation, or

the extemporaneously prepared admixture under study.

2. Materials and methods

The light obscuration (LO) and laser diffraction (LD) instruments were compared for the purpose of assessing their respective abilities to identify changes in the large diameter tail (i.e. >5 μm) of the GSD of lipid emulsions. The LO method (range: 1.3–400 μm, nominal), employing the SPOS technique, was used to determine the volume-weighted percentage fat, PFAT, contained in globules larger than 5 μm (LO '1' threshold of detection: 1.79 μm). As a particle or droplet in the applicable size range passes through an optical sensing zone that traverses the flow channel, a small fraction of the incident light beam is refracted and/or scattered by the particle. This effect causes a momentary decrease in the light intensity reaching a detector located across the flow channel. The height, or magnitude, of the resulting pulse (representing the change in intensity) is proportional to the square of the droplet diameter, for droplets smaller than the width of the optical sensing zone. As long as the droplet concentration is below the 'coincidence limit' of the sensor (9000 particles per ml), each globule is counted and sized one at a time. We conducted this experiment with automatic adustment (Autodilution, Appendix A, '1') of the fat globule concentration set to approximately 1/3 of the nominal coincidence limit for the sensor (LE400-05SE). The flow rate was optimized during calibration of the instrument at 1 ml/s and run at this rate throughout the study. Earlier measurements were made at a range of concentrations that proportionately verified the same particle/globule number per ml or volume-weighted value (Appendix A).

The LD instruments use two distinctly different physical techniques in order to produce an approximate representation of the particle size distribution — in this case, the lipid droplet size distribution. For the larger droplets — i.e. diameter >1.2 μm (approximate) — Fraunhofer diffraction is the operative technique. It is used to estimate the distribution of larger fat globules from the spatial pattern of light produced in the near-forward direction. For globules of a given size, the phenomenon of diffraction yields a pattern of concentric rings of alternating intensity maxima and minima. The periodicity, or angular extent, of this pattern is inversely proportional to the diameter of the globules. In the case of the smaller fat droplets, comprising most of the overall distribution — i.e., diameter <1.2 μm (approximate) — the theory of Mie scattering is used to analyze the variation of the scattered intensity with the angle of detection, over a large range of angles. This angular variation is significant whenever the droplet diameter is not small compared with the wavelength of the laser light (typically 0.6–0.7 μm) and occurs as a consequence of the mutual interference of the individual waves scattered from different points within each fat droplet. The LD instruments were optimized to operate at levels that avoided multiple scattering effects that would distort particle/globule size distribution.

In the case of a fat emulsion, there is an uneven distribution of fat droplet sizes — relatively small ones where Mie theory applies, plus larger globules for which Fraunhofer diffraction theory must be used. Mathematical algorithms are, therefore, needed to 'invert' both the small-angle (diffraction pattern) and large-angle intensity data, so that the resulting droplet and/or globule size distribution information can be smoothly combined. The ultimate goal of a LD-based instrument is to produce an accurate overall droplet/globule size distribution, ideally spanning a range from 0.05–0.1 μm to 50 μm, or larger, depending on the quality and stability of the fat emulsion in question. It must be emphasized that all LD instruments employ device-specific data inversion algorithms in order to calculate an overall fat droplet distribution, spanning all droplet/globule sizes. Owing to the 'ensemble' nature of the LD approach, requiring the use of relatively complex and proprietary data-inversion algorithms, different distribution should be expected from different instruments. Hence, in this study we tested the response of more than one LD instrument — identified here as LD1 '2', LD2 '3' and LD3 '4'. By contrast,

only one LO '1' instrument was used where the signal (change in light intensity) is due to the passage of each particle one at a time, and therefore, does not require complex data-inversion alogorithms. Rather, the single pulse heights are converted to diameters using a standard calibration curve constructed from monosized polystyrene microspheres of known dimensions ranging from 1.33 to 200 μm.

In this study, we conducted three separate experiments. In the first experiment, we validated sizing and counting accuracy of the LO method and instrument employing the SPOS technique, using 128 diameter channels with standard polystyrene (latex) uniform microspheres of known sizes. Specifically, 2 μm 5, 5 μm 6, 10 μm 7 and 25 μm 8 size standards, traceable to the National Institute of Standards and Technology (NIST), were used. With the exception of the 2 μm latex spheres, all size standards were automatically diluted by the LO instrument during the analysis. Due to the extremely high concentration of the 2 μm latex spheres, this size standard was first manually pre-diluted gravimetrically in a 19:1 water:size standard mixture (corrected for density differences), yielding a final theoretical concentration of 2.5×10^7/ml. This mixture was then treated like the others during the LO validation testing. Six replicates were analyzed to assess the numerical recovery of the stated concentrations for each nominal size standard. This experiment was not conducted using the LD instruments, as such quantitative validations based on absolute particle number are not possible.

In the second experiment, we tested the responses of the LO and LD instruments to a parenteral admixture containing unstable fat emulsion. The composition of the standard intravenous nutritional admixture studied was made from commercial ingredients '9' '10' and is shown in Table 2. A 3:1 volume ratio of solution-to-emulsion comprised the final admixture for study, resulting in a low osmolality formulation that might be given in a clinical setting to patients by either peripheral or central vein infusion. The admixtures were allowed to degrade with time, such that substantial growth of fat globules associated with the upper size range of the GSD occurred to varying degrees with aging. The admixtures were prepared at various times prior to the start of measurements, extending from just before the start to as long as 64 h before analysis, representing a range of elapsed-time intervals (0, −12, −18, −24, −40, and −64 h). Five representative PFAT (>5 μm) levels spanning the widest range of values, and therefore, instrument responses, were used for analysis. This approach ensured that a wide range of concentrations of coalesced fat globules existed in the large-diameter tail of the GSD. Given our earlier experience, (Driscoll et al., 1995, 1996, 1999, 2000a,b) admixtures found to have a PFAT (>5 μm) value of 0.4% or higher were considered pharmaceutically unstable.

Table 2
Final composition of the emulsion admixture studied

Component	Concentration (g/l)
Crystalline amino acids (8 essential and 7 non-essential)	22.5
Glycerin USP (glycerol)	22.5
Electrolytes:	
Sodium acetate•3 H_20 USP	1.5
Magnesium acetate•4 H_20	0.41
Calcium acetate•H_20	0.19
Sodium chloride USP	0.9
Potassium chloride USP	1.13
Phosphoric acid NF	0.3
Potassium metabisulfite NF	<0.37
Lipid emulsion:	
Safflower oil	25
Soybean oil	25
Egg phosphatides	3
Glycerin	6.2
Water for injection USP	quantum satis

Finally, in the third experiment we assessed the responses of the LO and LD instruments against an identical, but freshly-prepared, parenteral nutrition admixture containing stable fat emulsion that included varying concentrations of 5 μm latex standard particles. In order to achieve a two-log concentration range of latex mixtures, a more concentrated form of size standard was used '11', compared with the validation studies in the first experiment. The commercial concentration of 5 μm standards was progressively diluted with

nearly particle-free pharmaceutical water for injection. Each mixture was made by addition of a fixed number of latex spheres, as a fraction of the commercial concentration '11'. The latex-lipid mixtures were thus constructed so as to contain a known volume-weighted percentage of mass (latex + fat), or 'PMASS', calculated from:

$$\text{PMASS}(\%) = \left[V_S \times \left(\frac{N_{TOT}}{M_{TOT}} \right) \times 100 \right] + \text{PFAT}_{LO}$$

where, V_S = sphere volume of a 5 μm latex particle = $6.55 \times 10^{-11}/\text{cm}^3$, N_{TOT} = theoretical total number of latex spheres added, M_{TOT} = Total mass of FAT and Latex (g/ml), PFAT_{LO} = measured PFAT (> 5 μm) before addition of latex spheres = 0.012% (based on five separate measurements on different occasions, $n = 12$ samples)

Finally, in order to accommodate the finite width of the latex particle size distribution (and agglomerates), plus differences in the output formats of the four instruments, the threshold diameter was set at 2.30 μm. In this way, all particles larger than this size were accounted for in the final values. The resulting increase in the % volume of latex particles plus emulsion globules using this lower threshold, compared with the corresponding value obtained using the 'normal' 5 μm threshold, was negligible.

These emulsion 'spiking' studies were performed in order to simulate the growth in the large diameter tail of the GSD, as would occur in an unstable lipid emulsion over time. The same emulsion admixture was used as in the stability experiment. However, only stable emulsion (i.e. immediately following aseptic preparation) was used during this phase of the study, so as to allow assessment of each instrument's response to the addition of latex spheres of known size and concentration. Table 3 provides the concentrations of the 5 μm latex spheres used to spike the admixture tested.

These latter two experiments for evaluating the responses of the LO and LD instruments provided assurance that each sizing method was adequately studied. For each of the lipid emulsion stability assessments, the LO technique was used simultaneously during each LD test. This was accomplished on two separate occasions. A duplicate sample was taken from each emulsion admixture and the resulting two samples were simultaneously analyzed by both the LO and LD instruments at every stage of the study.

The results for the first experiment report the total number of latex particles measured. For the differential assessments in instrument performance in experiments 2 and 3, data were expressed as the volume-weighted portion of the particle/fat globule size distribution. The data generated by the LO, LD1 and LD2 instruments for each assessment were provided as volume-weighted results. In the case of the LD3 instrument, the volume-weighted data were obtained by integrating the values of the cumulative volume-weighted distribution (versus particle diameter) provided in the instrument printout.

3. Results

In the first experiment, the data for the numerical validation of the LO method using the various latex size standards appear in Table 4. In each case, the recovery of latex spheres on a number/ml basis was within 10% of the theoretical concentration stated in the documentation provided with each NIST size standard. Fig. 1 depicts the individual responses ($n = 6$ per size) of the LO assessment for the four size standards that were used for the validation experiment.

Table 3
Concentrations of 5 μm polymer microphere mixtures in the emulsion admixture studied

Formulation Number.	Concentration of 5 μm (particles/ml)[a]	% Solids
A	2.26×10^5	0.00155
B	4.51×10^5	0.00310
C	1.13×10^6	0.00777
D	2.26×10^6	0.01553
E	4.51×10^6	0.03106
F	2.26×10^7	0.15534
G	3.39×10^7	0.23301
H	3.94×10^7	0.27080

[a] Starting Latex standard concentration = 0.31% solids; calculated particle # /ml = $4.51 \times 10^7/\text{ml}^k$.

Table 4
Results of numerical validation of the LO instrument using NIST standard reference materials

Nominal Size	Number	[Theoretical per ml]	[Measurement per ml]	% Error
2 μm	6	2.5×10^7	$2.37 \pm 0.026 \times 10^7$	5.1 ± 1.1
5 μm	6	1.0×10^7	$1.09 \pm 0.001 \times 10^7$	9.4 ± 0.1
10 μm	6	1.0×10^6	$1.03 \pm 0.013 \times 10^6$	3.5 ± 1.3
25 μm	6	3.0×10^5	$3.00 \pm 0.023 \times 10^5$	0.5 ± 0.5

In the second experiment, the responses of each instrument, as the volume-weighted percentage of fat, PFAT > 5 μm, are tabulated in Table 5. As expected, admixtures with a PFAT > 5 μm of 0.4% or higher exhibited visual evidence of phase separation (free oil) in the majority of cases. As the instability studies were conducted on two separate occasions, the data are shown separately based on the volume-weighted results for the admixtures studied on different days (test periods # 1 and 2). The data presented were taken from the results of each instrument for the large diameter tail of the GSD for the lipid emulsions (all fat globules present > 5 μm) for all analyses. Compared with the LO data obtained during each study, the LD instruments provided highly variable results between instruments. For LD1, no globules were detected larger than 5 μm for the first three lipid emulsions, despite measurably different results obtained from the LO instrument (including in one case a level of PFAT > 5 μm exceeding 0.5%). At the extreme levels of emulsion instability depicted in the fourth and fifth emulsions, LD1 identified the presence of large populations of enlarged fat globules. The PFAT > 5 μm result was nearly comparable to that found by LO in the fourth emulsion, but was grossly overestimated in the fifth and most destabilized product.

The results comparing the LD2 and LD3 instruments were similarly incongruent with respect to the LO results. Although these instruments were able to detect enlarged fat globules in all of the emulsions tested, they overstated the volume-weighted fat percentage above 5 μm in every case, often by at least a factor of 10. For example, even though the first two emulsions would be considered well within the limits of our definition of pharmaceutical stability — i.e. PFAT (> 5 μm) < 0.4% — both LD instruments would have rejected the second emulsion by a very large margin (i.e. PFAT = 3.7 and 12%, respectively). The responses of each instrument to the tail of the GSD of each emulsion are graphically demonstrated in Fig. 2. In addition, for illustrative purposes, the full GSD result for each LD instrument is also depicted in Fig. 3. The LO instrument could not be included in Fig. 3, because its threshold of detection excluded lipid droplets smaller than 1.75 μm — i.e. the majority of the lipid droplet population (on both a number and volume basis).

In the final experiment, the responses of all the instruments to increasing concentrations of 5 μm latex spheres were assessed. Again, all data are expressed as volume-weighted results; however, in this case the total percentage of the mass includes fat globules plus latex spheres. Table 6 summarizes the parameters used to calculate the final results. Clearly, as the concentration of latex spheres increased, this component became increasingly significant compared with the nominally constant concentration of fat globules (eventually overwhelming the latter) in these stable lipid emulsions. Table 7 depicts the different results obtained by each of the instruments compared with the expected final results. Fig. 4 presents the volume-weighted results taken from the data in Table 7. Fig. 5 provides a numerical depiction of the data derived from the LO instrument.

4. Discussion

The infusion of particulate matter that exceeds the internal diameter of the pulmonary capillaries increases the risk of embolic syndrome. The clini-

cal risk associated with the intravenous infusion of rigid matter, such as large solid crystals, is generally acknowledged. One of the most dramatic clinical examples of the devastating physiological consequences of the infusion of crystalline precipitates was described in the April 18, 1994

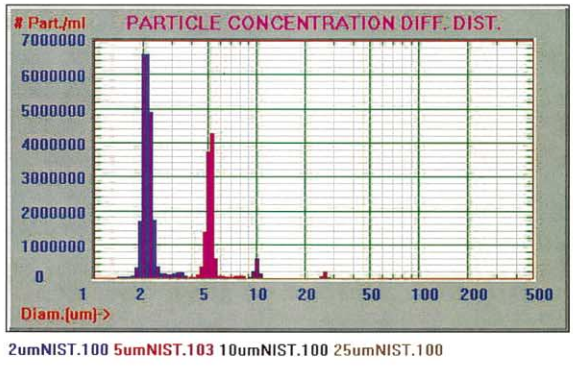


Fig. 1

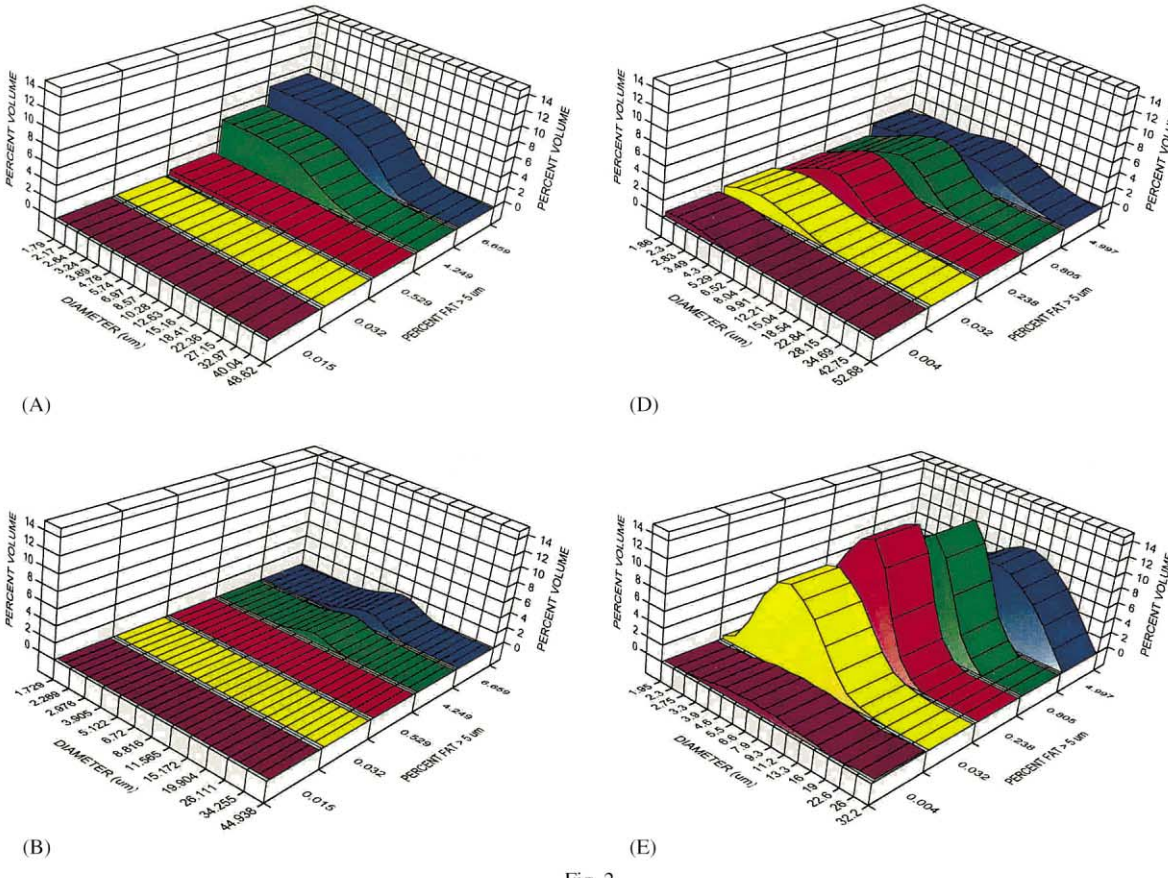
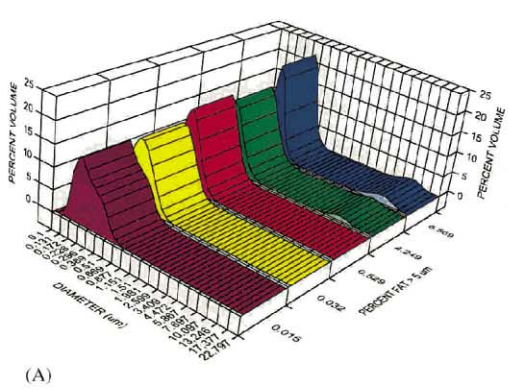


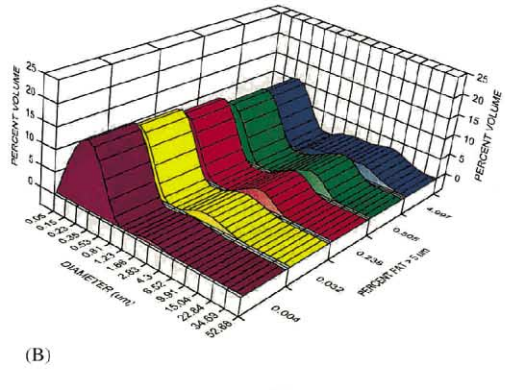
Fig. 2

Fig. 1. Population distributions from the numerical validation of the LO instrument.

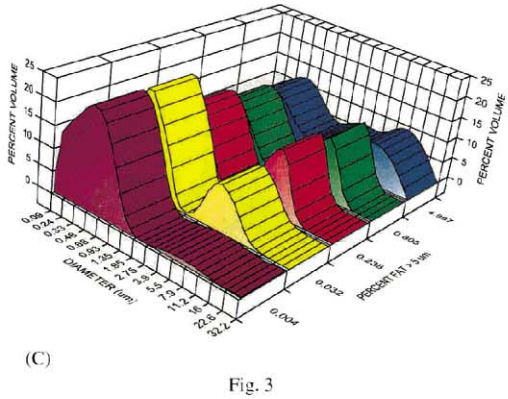
Fig. 2. LO and LD studies of the large diameter tail of the GSD of unstable lipid emulsion, (a) LO Depiction in Test period #1, (b) LD1 Depiction in Test Period #1, (c) LO Depiction in Test Period #2, (d) LD2 Depiction in Test Period #2, (e) LD3 Depiction in Test Period #2.



(A)

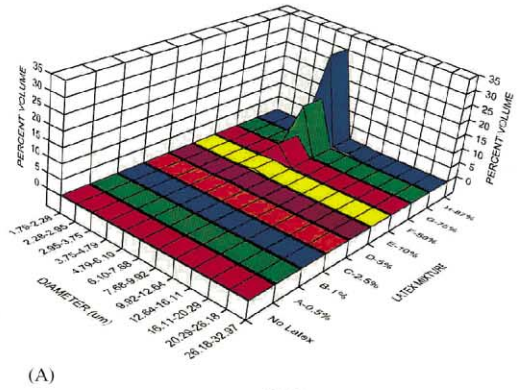


(B)

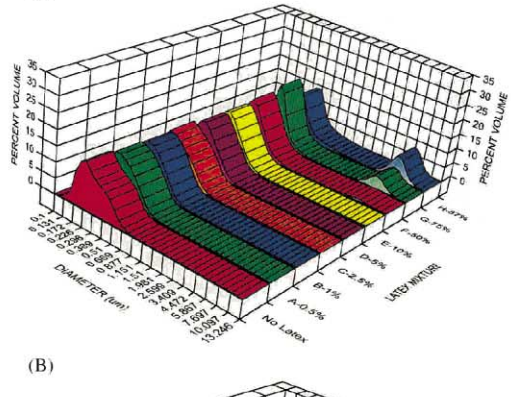


(C)

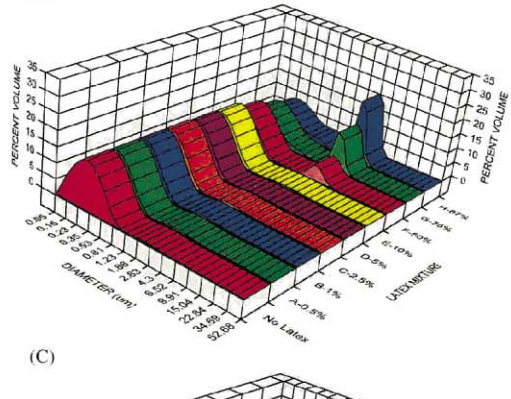
Fig. 3



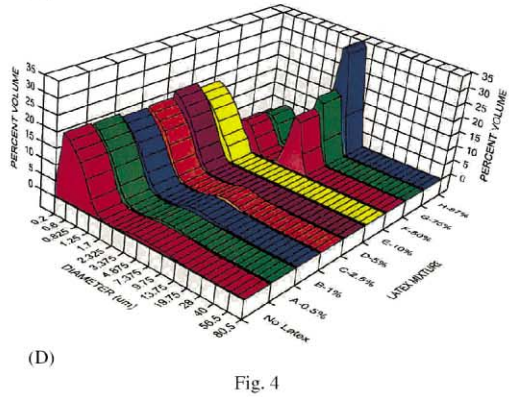
(A)



(B)



(C)



(D)

Fig. 4

Fig. 3. LD Studies of the Entire GSD of Unstable Lipid Emulsion, (a) LD1 depiction in Test Period # 1, (b) LD2 depiction in Test Period # 2, (c) LD3 depiction in Test Period # 2.

Fig. 4. LO and LD studies with latex spiking into stable lipid emulsion, (a) LO depiction of latex spiking, (b) LD1 depiction of latex spiking, (c) LD2 Depiction of Latex Spiking, (d) LD3 depiction of latex spiking.

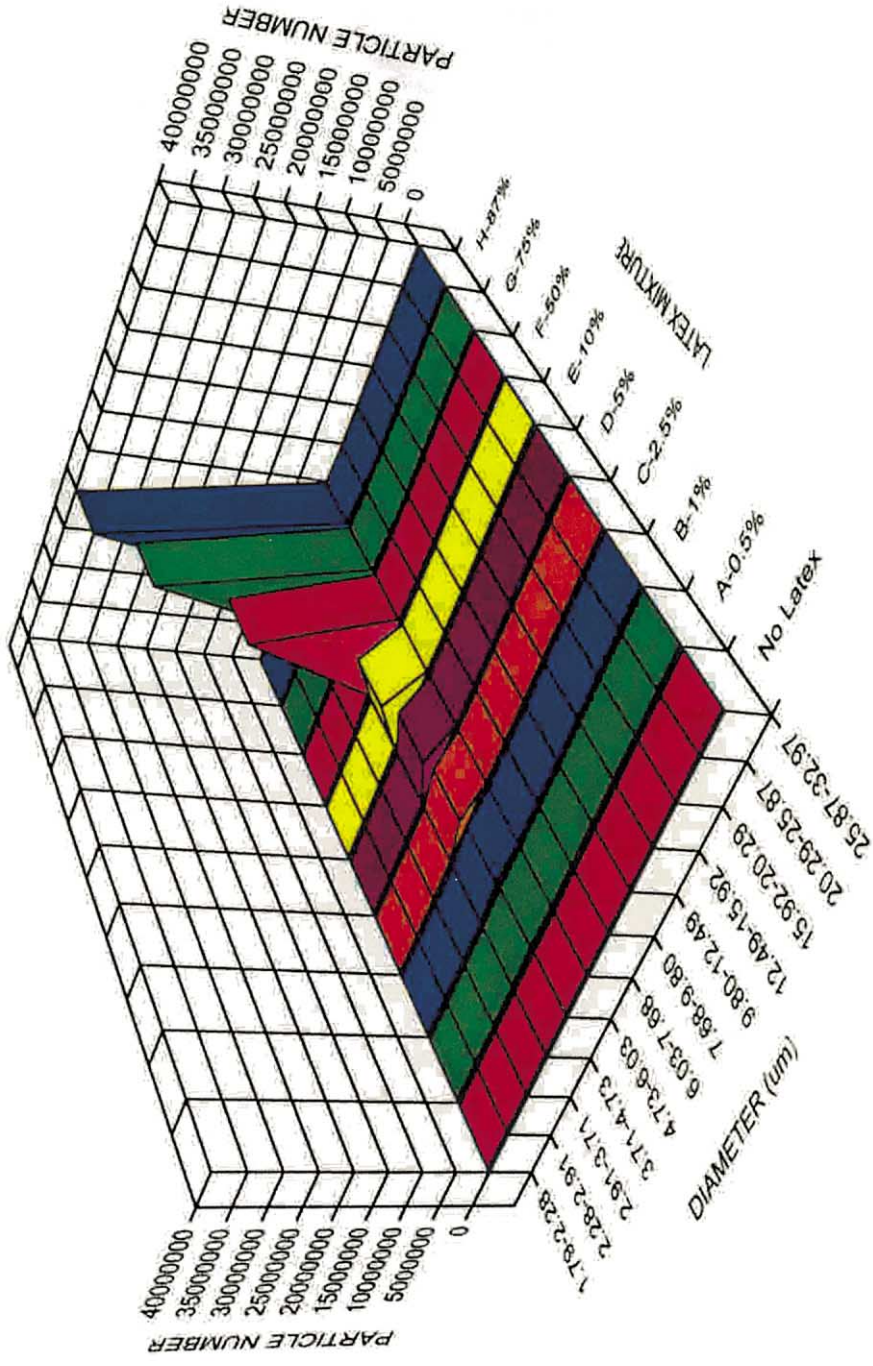


Fig. 5. Numerical depiction of LO results of latex spiked stable lipid emulsion.

Table 5
Volume-weighted percent fat in the large diameter tail of the globule size distribution

	Instruments			
PFAT >5 μm[a]	LO	LD1	LD2	LD3
Test Period #1	0.015	0.000		
	0.032	0.000		
	0.529	0.000		
	4.249	4.893		
	6.569	10.84		
Test Period #2	0.004		0.170	0.028
	0.032		3.690	11.98
	0.238		8.555	23.57
	0.805		17.39	29.27
	4.997		20.65	31.98

[a] Percent of fat present greater than 5 μm for duplicate samples.

Fig. 2. LO and LD studies of the large diameter tail of the GSD of unstable lipid emulsion, (a) LO Depiction in Test period #1, (b) LD1 Depiction in Test Period #1, (c) LO Depiction in Test Period #2, (d) LD2 Depiction in Test Period #2, (e) LD3 Depiction in Test Period #2.

FDA Safety Alert. Two patients died and at least two additional patients suffered a near-fatal respiratory injury from the infusion of an incompatible combination of parenteral calcium and phosphate salts (Food and Drug Administration, 1994).

The clinical risks associated with the infusion of flexible fat globules that have coalesced through colloidal destabilization are less clear. Nevertheless, we have shown that when the population of enlarged, unstable fat globules has grown sufficiently to yield PFAT > 5 μm values comparable of 0.4% or greater, the emulsion exhibits obvious physical signs of phase separation (i.e. free oil) and therefore, can be considered pharmaceutically unstable. Furthermore, given the physiological dimensions of the pulmonary microvasculature, such emulsions should be considered unfit for intravenous infusion.

Every effort should be made by the formulator of intravenous lipid emulsions to minimize the presence of oversize fat globules in the large diameter tail of the GSD, especially for extreme sizes above 5 μm that may have physiological implications. These enlarged fat globules are invariably present to a small extent in all formulations, however, any sudden increase in their numbers indicates active coalescence. Factors that initiate such adverse changes in the GSD can arise from environmental and end-user conditions. For example, elevations in storage temperature, particularly above 30°C, or mechanical agitation, as in the commercial transport of such products over long distances, are environmental variables that can compromise stability. Factors affecting lipid emulsion stability influenced by end-users, such as the introduction of oxygen upon opening the commercial product for intravenous infusion, or the addition of other nutrients such as amino acids, carbohydrates and electrolytes to form a parenteral nutrition admixture, will also affect stability.

Table 6
Percent of Solids in 5 μm Latex-Spiked Lipid Emulsion Admixtures

Mixture([a])	[Latex]	Latex-%	Fat-%	Total-% Mass
Emulsion alone	0/ml	0.00000	5.000	5.000
A (0.5%)	2.26×10^5/ml	0.00155	4.975	4.976
B (1.0%)	4.51×10^5/ml	0.00310	4.950	4.953
C (2.5%)	1.13×10^6/ml	0.00777	4.875	4.883
D (5.0%)	2.26×10^6/ml	0.01553	4.750	4.765
E (10.0%)	4.51×10^6/ml	0.03106	4.500	4.531
F (50.0%)	2.26×10^7/ml	0.15534	2.500	2.655
G (75.0%)	3.39×10^7/ml	0.23301	1.250	1.483
H (87.0%)	3.94×10^7/ml	0.27080	0.633	0.904
[a]Latex 4205A (100%)	4.51×10^7/ml	0.31000	0	0.310
Latex 4K-05 (100%)	1.00×10^7/ml	0.06900	0	0.069

[a] Mixtures A–H are dilutions from Latex 4205A and are also listed () as a percentage of the starting concentration.

Table 7
Volume-weighted percent of solids in 5 μm Latex-spiked lipid emulsion admixtures

Mixture[a]	Vol-% Expected	Vol-% Measured (Fat + Latex)[a]			
		LO	LD1	LD2	LD3
Fat w/o Latex	<0.4[b]	0.012	0.000	0.700	6.784
Fat w/0.5% Latex	0.042	0.035	0.000	1.292	6.391
Fat w/1.0% Latex	0.072	0.073	0.000	1.362	9.322
Fat w/2.5% Latex	0.163	0.186	0.000	1.282	8.642
Fat w/5.0% Latex	0.323	0.371	0.000	1.047	0.000
Fat w/10% Latex	0.663	0.753	0.000	1.857	0.000
Fat w/50% Latex	5.586	5.649	2.488	13.287	47.919
Fat w/75% Latex	14.981	15.374	14.800	29.892	57.661
Fat w/87% Latex	28.551	30.199	28.402	43.837	98.238
Overall Error (%)		8.77 ± 6.66	72.97 ± 43.46	1320 ± 1931	323.36 ± 438.16

[a] All samples performed in duplicate.
[b] The freshly-prepared, starting admixture without latex added was expected to be stable, and therefore, with a value of PFAT (>5 μm) less than 0.4%.

Methods of assessing the consequences of these common factors must be able to detect changes in the upper size range of the GSD (i.e. >5 μm). Specifically, they must not only offer qualitative information about the GSD, but also provide an accurate quantitative assessment of emulsion quality. Presently, only the LO method using the SPOS technique offers this capability in a manner that is easily applied, does not introduce additional stability issues, and yields statistically valid results that closely depict the GSD for these highly concentrated dispersions. First, the LO/SPOS method is much more sensitive than the LD method with respect to detection of enlarged fat globules in the upper 'tail' of the GSD in unstable fat emulsions. This conclusion should not be surprising, given the fact that the LO/SPOS method, like ESZ, is insensitive to the vast majority of submicron (mean of 0.3 μm) fat droplets comprising the GSD for $>10^{13}$ droplets per ml. This attribute allows it to 'focus' on the very small fraction of enlarged, potentially significant fat globules that comprise the upper tail of the GSD. The fact that the LO/SPOS method responds to fat globules one at a time allows it to possess, in theory, the highest possible accuracy and resolution, yielding GSD results that are virtually devoid of computational artifacts. By contrast, the LD method is necessarily much less sensitive to small changes in the upper tail of the GSD associated with enlarged fat globules, owing to the ensemble nature of the measurement. Fat droplets/globules of all sizes contribute to the overall diffraction/scattering 'signal', requiring, therefore, an inversion algorithm to establish a droplet size distribution. While LD instruments are able to signal the presence of enlarged fat globules if the latter are present at sufficiently high concentrations, these devices have been found consistently to lack the sensitivity necessary to validate lipid emulsion stability. In addition, these instruments clearly appear unable to achieve either the accuracy or the resolution that can be routinely obtained by methods that individually count and size fat globules. Without the ability to actually count the enlarged globules, it is impossible to accurately provide critical details of the large-diameter tail in the GSD. This deficiency was clearly evident in this study.

Finally, it should be noted that the only measurement method which can even approach the LO method with respect to sensitivity, accuracy and resolution is ESZ. However, the LO method possesses a number of quantitative and qualitative advantages over ESZ. The principal advantage is significant statistical improvement in the resulting GSD results, owing to the fact that an LO instrument is typically able to count/size fat droplets at

a rate 40–50 times higher than that achieved by an ESZ instrument. Therefore, the actual volume of original fat emulsion typically sampled in an LO/SPOS measurement over a given analysis interval is substantially larger than that obtained by ESZ by the same factor. This difference has led to the recent development of a quantifiable pharmaceutical definition of IVLE stability through the use of the volume-weighted percentage of fat (PFAT) in the upper size region of the GSD (Driscoll et al., 1995).

In the first experiment, the validation studies for the recovery of latex size standards demonstrated the quantitative accuracy of the LO technique. Although the latex suspensions are size standards, their concentrations are within 10% of the manufacturer-stated amounts; thus, the size standards may also be viewed as acceptable number standards. The $<10\%$ error observed here exceeds the specifications stated in Chapter ⟨788⟩ of the USP, and therefore, the LO specifications set forth by the USP may need to be revised. Given the potential physiological importance of particulates or globules in the systemic circulation, a higher standard should be adopted for LO validation if possible. In the second and third experiments, the LO-derived data became the standard with which the responses of the LD method were compared during the assessment of the stability of IVLEs.

In the second experiment, the LO method consistently distinguished the difference between a stable and unstable emulsion according to our criterion, PFAT (>5 μm) $\geq 0.4\%$ of the total fat present. An example, using the most unstable emulsion studied, appears in Fig. 6 that depicts the coarseness of the GSD. The LD1 data were least sensitive to the changes in the large diameter tail of the GSD when PFAT (>5 μm) was less than 1%. Both the LD2 and LD3 results grossly overestimated the value of PFAT (>5 μm) by a factor of at least 10 in all cases compared with the LO analysis results.

In the third and final experiment, as in the first experiment, the LO data were within 10% of the expected volume-weighted result for the recovery of the latex spheres. As in the second experiment, the LD1 instrument was least sensitive to the presence of 5 μm particles; only when the percentage reached almost 6% were they detected. Similarly, the response of the LD2 instrument to the latex-spiked emulsions also overestimated the vol-

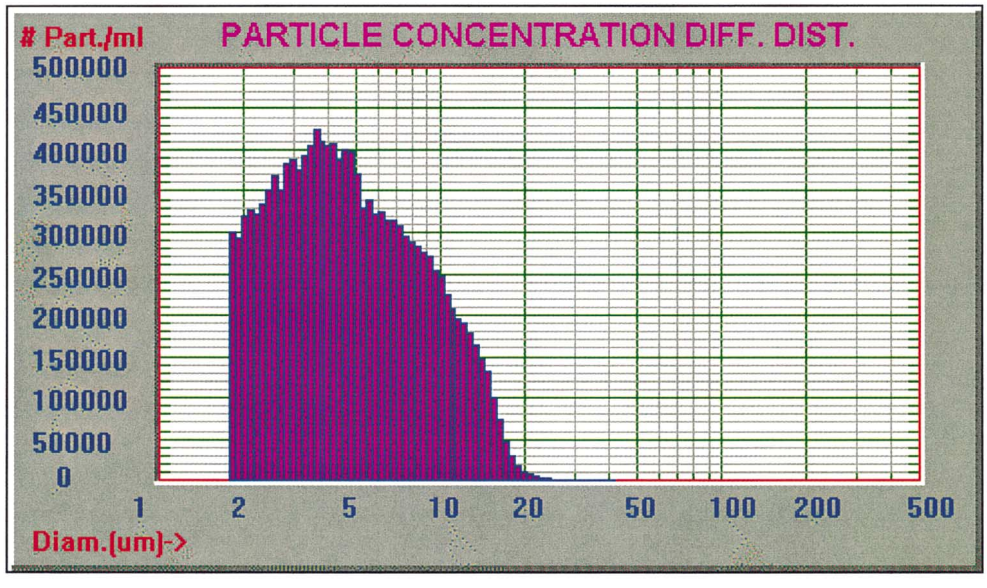


Fig. 6. Coarseness of the globule size distribution in the admixture with a PFAT >5 μm $= 6.659\%$.

ume-weighted PFAT (>5 μm) results. Finally, the LD3 instrument was inconsistent, missing altogether the presence of latex spikes at intermediate concentrations, despite detection at lower and higher concentrations of the added latex standards.

Recently, a number of methods have been compared which assess the stability of all-in-one mixtures, applying various forms of assessment as outlined in the most recent revision of the USP proposed Chapter ⟨729⟩. In 1992, Washington and Sizer tested the stability of all-in-one mixtures, comparing LD and ESZ (Washington and Sizer, 1992). They concluded that the LD results were less sensitive than the data obtained by ESZ on the same admixtures, pointing out the value of single-particle electrical-zone sensing technique for all-in-one mixture stability studies. In 1995, Driscoll et al. compared the admixture stability results obtained from LO with those found using PCS (Driscoll et al., 1995). PCS resembles LD, in that particles of all sizes contribute to the 'signal' that is detected, requiring a complex mathematical algorithm to convert the raw data to a size distribution. Therefore, it is far less sensitive than LO in its ability to detect unstable emulsions. Only after the PCS data were stratified according to the corresponding LO value of PFAT >5 μm, either $<0.4\%$ or $>0.4\%$, was there a significant correlation with admixture stability. Finally, in 1996 Koster et al. evaluated qualitatively the differences in the sensitivity of detection of fat globules for the LO and LD methods in the upper size range of the GSD (Koster et al., 1996). Although these mixtures appeared to be stable, they showed that the LD method did not indicate the presence of any fat globules larger than 1 μ. By contrast, the LO method detected particles larger than 5 μm in all lipid mixtures. Our data confirm the inaccuracies of the LD method when compared with single-particle zone sensing techniques, such as the LO method, for both stable and unstable lipid mixtures.

Finally, our data suggest that IVLEs should have specific limits for an acceptable GSD that meets pharmacopeial standards. For commercial IVLE from the manufacturer, we would suggest a mean droplet size (MDS) that does not exceed 450 nm, and an upper limit for PFAT >5 μm that does not exceed 0.05%. Based on our experience and the data shown in Table 1, these limits are easily met. Therefore, such a proposed range for both MDS and PFAT (>5 μm) is pharmaceutically reasonable, in that the ranges are not only sufficiently broad, but also are likely safe, given current use conditions and available data.

For extemporaneously prepared all-in-one mixtures or other clinical modifications of commercial IVLEs, the same MDS applies. However, in these mixtures, because extemporaneous manipulations initiate instability, the upper size range limit for PFAT >5 μm is approximately one log higher (i.e. PFAT >5 μm $<0.4\%$) than our recommended limit for commercial IVLEs. This limit would apply to a beyond-use date assigned by the clinician.

5. Conclusions

This report indicates that the LO/SPOS provides superior methodology for the detection and enumeration of globules >5 μm in size for both commercial lipid emulsions and those extemporaneously compounded as all-in-one mixtures. Both ESZ and microscopic tests have the potential for quantitative assessments as well, but their use should ideally be correlated with studies conducted by LO/SPOS. Importantly, this study describes a method using particle size standards, which can be applied to verify the counting and sizing accuracy of any method used to assess lipid emulsion stability.

The section of the proposed Chapter ⟨729⟩ of the USP that deals with quantitatively determining the presence of enlarged, coalesced fat globules in intravenous lipid emulsions must identify techniques that can recognize adverse changes in the large-diameter tail of the GSD (quantitate precisely globule sizes >1 μm). Enlarged fat globules in this region of the GSD may assume clinical significance. A second method that identifies the mean droplet size of the emulsion must be included in the proposed Chapter $<729>$. Inversion techniques such as PCS (based on analyzing the temporal fluctuations in the scattered

light intensity due to Brownian motion) or LD (based on analyzing the angular dependence of the scattered light intensity using Mie theory) would acceptably provide this latter information. Thus, the critical pharmaceutical assessments of any intravenous emulsion should always include two key parameters in order to characterize fully the GSD and meet pharmacopeial standards. These are: a submicron assessment, that depicts the mean droplet size, and a large-diameter measurement that characterizes the tail of the GSD. Without these two measurements, it is impossible to validate accurately and reproducibly the integrity and safety of any emulsion intended for intravenous administration. We also propose that the official monograph for IVLEs set specific limits for the GSDs for both commercial and extemporaneously prepared products.

Acknowledgements

Thomas M. Gonyon, Research Associate, Corporate Research and Technical Services, Baxter Healthcare Corporation, Round Lake, IL, USA Horst Peterss, Research Associate, Pharmaceutical Development, Hospital Care Division, B. Braun, Melsungen, Germany

Appendix A

1. AccuSizer 780/APS (version 1.59), Particle Sizing Systems, Santa Barbara, CA, USA
2. LA-920 for Windows (version.b2.01), Horiba Instruments, Irvine, CA.
3. MasterSizer X (version 1.2), Malvern Instruments Inc., Southborough, MA, USA
4. HELOS (H0869) Particle Size Analyzer, Sympatec Instruments, Clausthal-Zellerfield, Germany.
5. Certified Size Standards, (NIST Traceable) 2 μm (nominal size) polymer microspheres, 4K-02, 5×10^8/ml, Lot no. 20525 Duke Scientific Corporation, Palo Alto, CA, USA
6. Certified Size Standards, (NIST Traceable) 5 μm (nominal size) polymer microspheres, 4K-05, 1×10^7/ml, Lot no. 20535 Duke Scientific Corporation, Palo Alto, CA, USA
7. Certified Size Standards, (NIST Traceable) 10 μm (nominal size) polymer microspheres, 4K-10, 1×10^6/ml, Lot no. 20467 Duke Scientific Corporation, Palo Alto, CA, USA
8. Certified Size Standards, (NIST Traceable) 25 μm (nominal size) polymer microspheres, 4K-25, 3×10^5/ml, Lot no. 20428 Duke Scientific Corporation, Palo Alto, CA, USA
9. ProcalAmine Solution (3% amino acids and 3% glycerin injection with electrolytes), lot nos. J9A012 and J9B014, McGaw Labs, Irvine, CA, USA
10. Liposyn II 20% Lipid Emulsion, lot nos. 49–433-DE and 57–372-DE, Abbott Laboratories, North Chicago, IL, USA
11. Certified Size Standards, (NIST Traceable) 5 μm (nominal size) polymer microspheres, 4205A, 0.31% solids, Lot no. 21365 Duke Scientific Corporation, Palo Alto, CA, USA

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