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Expert Review

Lipid Injectable Emulsions: Pharmacopeial and Safety Issues

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Abstract. Lipid injectable emulsions have been routinely used in patients worldwide for over 40 years as a nutritional supplement in patients requiring parenteral nutrition. They can be given as a separate infusion or added into total parenteral nutrition admixtures. Despite such broad use, no pharmacopeial standards exist with respect to the optimal pharmaceutical characteristics of the formulation. Several attempts to establish standard physical and chemical attributes have been attempted by various pharmacopeias around the world, but without success largely due to technical issues regarding the creation of globule size limits. Recently, the United States Pharmacopeia has revised its previous efforts and developed two methods and criteria (under Chapter <729>) to measure the mean droplet size (Method I), and the large-diameter tail > 5 μm (Method II) of the globule size distribution to verify the stability of lipid injectable emulsions. Importantly, it is the latter size limits of Method II that have the greatest implications for infusion safety. The major safety issues involving lipid injectable emulsions include impairments in plasma clearance in susceptible patients, and the infusion of an unstable emulsion containing large quantities of potentially embolic fat globules. Recent animal studies investigating the toxicity from the infusion of unstable lipid injectable emulsions have shown evidence of oxidative stress and tissue damage to the liver when recommended globule size limits determined by Method II of the USP are exceeded. Adoption of Chapter <729> of the USP seems appropriate at this time.

KEY WORDS: globule size distribution; infusion container; lipid injectable emulsions; safety; volume-weighted $PFAT_5$.

INTRODUCTION

Lipid injectable emulsions, as soybean oil-in-water formulations stabilized by egg phospholipids, have been used worldwide in the clinical setting as a nutritional supplement for more than 40 years. More recently, an array of oils, individually or as mixtures, have been used in lipid injectable emulsions in addition to soybean oil, including safflower oil, medium chain triglycerides, olive and fish oils. Parenteral emulsions are commonly manufactured via a homogenizer that forces a concentrated oil–emulsifier–water mixture through a small orifice at very high pressures. This action is repeated through several cycles and subjects the mixture to very high shear forces, ideally producing a fine dispersion of submicron droplets with a narrow distribution, or low degree of polydispersity. There are three basic types of pharmaceutical emulsions which can be classified according to their mean droplet size (MDS): (1) a 'micro-emulsion', MDS <0.1 μm, such as liposomal drug formulations; (2) a 'mini-emulsion', MDS <1.0 μm, such as lipid injectable emulsions; and (3) a 'macro-emulsion', MDS >1 μm, such as chemoembolization infusions. The micro-emulsion is transparent, forms spontaneously and is thermodynamically stable, whereas the latter two are turbid, require energy to be formed, and are thermodynamically unstable.

The clinical use of lipid injectable emulsions in the US has spanned approximately 30 years. They have been principally indicated for patients requiring parenteral nutrition support as a source of essential fatty acids (i.e., linoleic and linolenic acids), and as a dense source of 'isotonic calories'. More recently they have been used as drug delivery vehicles for poorly soluble drugs such as anesthetic/sedative, propofol. They can be administered via the small veins of the peripheral venous circulation, i.e., relatively low-flow blood vessels (e.g., basilic or cephalic veins) that are physiologically limited by the final tonicity of the infusion. In general, peripheral vein tolerance is achieved for reasonable periods of time (usual intravascular catheter life: up to 72 h), before phlebitis inevitably occurs, as long as the osmolarity is kept between 600 and 900 mOsm/l (1,2). Higher osmolarity (e.g., 1,000–3,000 mOsm/l) formulations, as encountered in the preparation of total parenteral nutrition admixtures, can be administered for prolonged periods (usual intravascular catheter life: 7–10 days in the hospital, to years in the home infusion setting) via large veins of the central venous circulation (e.g., via the subclavian vein with radiographic confirmation of the catheter tip in the superior vena cava or SVC). The large volume of blood flow through the SVC represents approximately one half of cardiac output or about 2,500 ml/min, so catheter tip placement in this vessel provides maximal hemodilution of the infusate. Hence, lipid injectable

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emulsions may also be administered via these high-flow vessels as well. For most patients requiring total parenteral nutrition (TPN), where the osmolarity is very high from the combination of 15–18 crystalline amino acids, dextrose, 10–12 electrolytes, 12–13 multivitamins and 5–7 trace minerals, the use of central venous catheters to deliver this therapy, especially for prolonged periods of time, is mandatory. In addition, 20% soybean oil lipid injectable emulsions are commonly added to these TPN formulations (known as all-in-one or total nutrient admixtures), often comprising up to 30% of the total caloric intake. Adding lipids transforms the conventional parenteral solution to an emulsion, thus engendering potentially significant stability issues arising from the numerous ionically active components typically found in these extemporaneously prepared infusions.

The United States Pharmacopeia (USP), whose mission statement includes that it "promotes the public health and benefits practitioners and patients by disseminating authoritative standards and information" (3), is responsible for creating official articles (e.g., drug monographs and chapters) that are recognized and enforceable by the Food and Drug Administration (FDA). Under usual circumstances, the goal of the USP is to generate an official drug monograph within 3–5 years after its FDA approval (personal communication, Roger Williams, Executive Director, USP, October 24, 2000). With respect to lipid injectable emulsions, the USP began its first pharmacopeial preview in 1991 of a monograph entitled "Intravenous Fat Emulsion" (4) and the associated chapter <728> entitled "Globule Size Distribution in Intravenous Emulsions" (5), approximately 15 years after their introduction into the US for clinical use. Several 'in-process' revisions followed in 1994, 1995 and 1998, without adoption of an official monograph or chapter. The task was referred to another USP expert committee in 2000, resulting in the publication of another monograph in 2003. Unfortunately, like the previous versions, no globule size limits were suggested. However, without globule size limits and the appropriate instrumentation to define them, the essential question relating to emulsion stability and subsequent safety, could not be answered. In 2004, the USP took a different approach and decided to first publish a new version of chapter <729> (formerly <728>) entitled "Globule Size Distribution in Lipid Injectable Emulsions" (6) which detailed two methods of globule size analysis with specific pharmacopeial limits. Since then, a second chapter revision has been published in 2005 (7) along with a new monograph entitled "Lipid Injectable Emulsions" in 2005 (8) and again most recently in 2006 (9), that incorporates the proposed globule size limits.

There are two goals in this review. The first will be to detail the key issues regarding the proposed globule size limits of USP <729> with respect to the physical stability of lipid injectable emulsions, both in the native state and after inclusion in the TPN bag as a total nutrient admixture (TNA). The second goal, as a logical extension of the aforementioned stability issue, will be to discuss the implications of globule size for infusion safety.

PHARMACOPEIAL ISSUES AND LIPID INJECTABLE EMULSIONS

The current edition of USP chapter <729> (7) and its accompanying monograph (9) makes clear the desirable physical and chemical characteristics of lipid injectable emulsions in meeting pharmacopeial standards. Destabilization of these mini-emulsions via coalescence is the inevitable outcome of these thermodynamically unstable dosage forms. The goal of the USP in outlining the globule size limits is to ensure that the dosage form does not prematurely progress to a stage where the process of coalescence advances to a critical point before the end of its shelf-life, where the safety of the infusion is compromised (i.e., the formation of potentially embolic fat globules larger than 5 μm). There are a variety of common physical and chemical stresses that can accelerate the destabilization process. In one case, destabilization is inadvertent and insidious (e.g., during transportation and storage). US manufacturers of lipid injectable emulsion explicitly state the required storage temperature for these products. They differ, however, in that one stores the product in a conventional glass bottle, while the other stores the lipid injectable emulsion in a newly introduced plastic bag. In addition, they also differ in their storage requirements. For example, in the case of the plastic product, it is recommended "...not to be stored above 25°C" (10), while with the glass product, it is recommended to be "...stored at room temperature (25°C); however, brief exposure up to 40°C does not adversely affect the product. Do not store above 30°C" (11). Alternatively, destabilization can also occur during manipulation of the dosage form by the clinician where, for example, the manufacturer's container is 'spiked' with an intravenous administration set, thereby breaking the sterile seal and exposing the contents to microbial contamination and possibly leading to destabilization of the emulsion. Alternatively, lipid injectable emulsions can be used to compound TNAs. From a stability standpoint, it is important to ensure that the necessary manipulations by clinicians to prepare and administer a lipid injectable emulsion do not cause it to become so unstable during the period of infusion that it produces pathophysiological consequences.

The most common lipid injectable emulsion used clinically is a soybean oil-in-water formulation. The soybean oil droplets are stabilized by an egg phospholipid emulsifier that coats the submicron droplets. The hydrophobic tails of the phospholipids, containing long-chain fatty acids such as the 18-carbon oleic acid, align at the oil droplet surface of the internal phase, while the hydrophilic heads, containing phosphatidic acid, projects out into the external water phase. At or near physiologic pH, the polar phosphate head groups are ionized which establishes electrostatic repulsion between neighboring charged droplets, thereby conferring stability to the formulation. The pharmacopeial limits specifically outline the physical and chemical attributes of the dosage form that should be maintained throughout its shelf-life. They mainly include the pH, free fatty acids concentration, and globule size limits, and these are listed in Table I.

Table I. Lipid Injectable Emulsions 10, 20 or 30% w/v

Physicochemical Attribute	Pharmacopeial Limits
pH	Between 6.0 and 9.0
Mean droplet size	≤500 nm
$PFAT_5$	≤0.05%
Free fatty acid	≤0.07 mEq/g

pH Limits

The proposed pharmacopeial pH range for lipid injectable emulsions is between 6.0 and 9.0, whether its intended use is for nutrition support or as a drug delivery vehicle, and this range should be maintained throughout its shelf-life. During the normal shelf life of lipid injectable emulsions (i.e., between 18 and 24 months), the initial pH is nearer 9.0, whereas at the end of its shelf life, it approaches 6.0. This is to be expected as a result of the hydrolytic degradation of the long-chain trigylceride to its constituent free fatty acids over time. As the pH drops, the stabilizing anionic electrostatic charge conferred to the droplets by the egg phospholipids moieties is reduced, as the latter become less ionized, thereby reducing the stability of the emulsion. In the extreme case, the effect of an increasingly acidic pH will eventually neutralize the electrostatic charge residing on the lipid droplets at a pH of 3.2 (12). Even with seemingly modest reductions in the proposed pharmacopeial pH range of a lipid injectable emulsion formulation, the detrimental effects on stability can be seen. For example, a generic 1% propofol formulation in 10% lipid injectable emulsion that is preserved with sodium metabisulfite, thus requiring a unique final acidic pH range (4.5–6.6), has been shown to be unstable during its manufacturer-assigned shelf-life, as evidenced by a growing (coalescing) large-diameter tail, measured by light obscuration or extinction employing a single-particle optical sensing (LE/SPOS) technique (13). In other cases, when the emulsion is extemporaneously compounded into TNAs for adults producing admixtures with a pH of approximately 5.7, the short-term exposure to these acidic conditions does not appear to adversely affect emulsion stability over the period of clinical use (i.e., up to 30 h at room temperature) (14). For physical mixtures of lipid injectable emulsions containing medium-chain triglycerides (MCTs) along with long-chain triglycerides (LCT) provided as soybean oil, physical stability has been maintained for up to 48 h in neonatal/infant TNA admixtures with final pH values as low as 5.0 (15). The stabilizing influence of MCTs when combined with LCTs as physical mixtures have been shown to produce more stable TNAs than those prepared from LCTs alone (16).

Free Fatty Acid Limits

Free fatty acid concentrations in lipid injectable emulsions reflect the relative stability of the phospholipid emulsifier due both to its hydrolysis during heat sterilization, as well as from the breakdown of the triglyceride over the normal time course of the shelf-life for these formulations (17,18), i.e., 18–24 months. The principal long-chain fatty acids found in soybean oil include (in decreasing order) linoleic, oleic, palmitic, linolenic and stearic acids. From a quantitative standpoint over time, the 10, 20 or 30%$_{w/v}$ triglyceride concentrations found in lipid injectable emulsions are major contributors to the formation of free fatty acids over time compared to the amounts in the egg phospholipids emulsifier (i.e., 0.74–1.8%$_{w/v}$)—hence, the pharmacopeial limit of $\leq$0.07 mEq/g largely pertains to changes in the stability of the triglyceride. Moreover, the free fatty acid limit is also based on some concerns of systemic toxicity. In dogs, parenteral administration of free fatty acids from hydrolyzed lecithin produced blood and liver abnormalities (19), while in rabbits, intravenous infusion of free fatty acids caused pulmonary edema and ventilatory defects (20). In fact, an oleic acid-induced lung injury model has been used in animals to evaluate potential treatments of lung injury in the critically ill (21). In humans, displacement of bilirubin by free fatty acids from serum albumin occurs (22), and may be a factor in the development of kernicterus in premature infants. In some cases, the dose of lipid injectable emulsions may need to be reduced by one half of the usual lipid dose until the bilirubin level is lowered (23). Thus, limiting the amount of free fatty acids in commercial lipid injectable emulsions is primarily intended as a measure to improve the stability of the main emulsion components, as well as to minimize exposure to these hydrolysis byproducts upon intravenous administration and reduce subsequent potentially adverse clinical consequences.

Globule Size Limits

In any emulsion, determination of stability is demonstrated by maintenance of the globule size distribution (GSD) within defined limits (i.e., no growth in the extreme population of large-diameter fat globules). Alterations in the GSD reveal a change in the stability of the lipid injectable emulsion. The destruction of an emulsion system is manifested by the fusion of droplets that ultimately separate from the dispersed phase as enlarged fat globules via a process known as coalescence. From a clinical perspective, globule size limits for lipid injectable emulsions are most important, as they ultimately reflect the safety of the formulation.

The USP proposes globule size limits using two methods, applying two criteria to measure the mean droplet size (MDS), and the large-diameter tail of the GSD to verify the stability of lipid injectable emulsions. The first of these measures, the intensity-weighted MDS, expressed in nm, is an important *qualitative* measure of the homogenization process, and the USP specifies that the MDS cannot exceed 500 nm, irrespective of the final concentration of the dispersed lipid phase (i.e., 10, 20 or 30%$_{w/v}$). The technique for determining MDS, as per Method I of USP <729>, can be accomplished using either dynamic light scattering or Classical Mie or 'static' light scattering. Clearly, for lipid injectable emulsions that meet USP <729> limits, the greatest mass of the dispersed lipid droplets resides below 1 μm, but assuming a normal or Gaussian droplet size distribution for stable lipid injectable emulsions, there will be droplet extremes on both the left- (sub-micron) and right-side (up to and above 1 μm) tails of the distribution. Assuming a normal distribution of droplets, Fig. 1 illustrates the relative distribution of droplet sizes in stable *vs* unstable lipid injectable emulsions. For example, if a stable lipid injectable emulsion has an MDS of 300 ± 60 nm ($\mu \pm \sigma$), the proportion of droplet sizes of 375 nm (x') or larger can be estimated by calculating its z-value, and then identifying the corresponding area under the (Normal Distribution) curve (AUC) from standard statistical tables. In the above example, the calculated z-value is:

$$z = \frac{x' - \mu}{\sigma} = \frac{375 - 300}{60} = 1.25$$

A z-value of 1.25 corresponds to AUC = 0.7887, with one half the remaining area of the curve lying to the right of

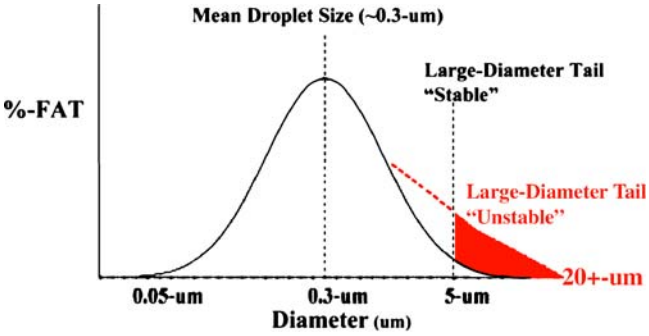

Fig. 1. Normal probability curve and relevant droplet/globule populations for lipid injectable emulsions*.
*Previously published from reference 26.

this, i.e., 10.5% of the droplets are above 375 nm, or, alternatively, 89.5% of droplets are below 375 nm. At 2σ (i.e., $x''' = 420$ nm) above the mean diameter, with an AUC = 0.9545, 2.28% of the droplets are above 420 nm. Finally, at 3σ (i.e., $x''' = 480$ nm) above the mean diameter with an AUC = 0.9973, 0.135% of MDS values are above 480 nm. Obviously, there are diminishing droplet numbers at either extreme of the GSD of stable lipid injectable emulsions. In particular, the population of large-diameter fat globules larger than 1 µm is vanishingly small, but nonetheless, they are certainly present. In 1980, the British Pharmacopeia attempted to set limits for lipid injectable emulsions and stated no fat globules could exceed 5 µm in size (24). This requirement, of course, was subsequently dropped, as it was based on flawed technological capabilities at that time, rather than actual measurements of statistically irrelevant, but potentially clinically significant, large-diameter fat globules. Method I of the USP <729> is not capable of discerning the large-diameter population of these statistical 'outliers', despite their pathophysiological significance.

Importantly, the use of light obscuration or extinction, employing a single-particle optical sensing (LE/SPOS) technique as described in Method II of <729>, has been shown to reproducibly measure this extreme globule outlier population in a series of commercially available, stable lipid injectable emulsions. (25) Moreover, this study also showed that the LE/SPOS technique could be validated in terms of sizing and counting accuracy using certified (traceable to the National Institute of Standards Technology) calibrator polymer microspheres. By comparison, laser diffraction showed nonlinear responses to the same lipid admixtures and varying concentrations of calibrator microspheres as shown in Fig. 2. (26) A summary of these findings and application of various sizing methods for lipid injectable emulsions were recently reviewed. (27) Method II of <729> specifies that the volume-weighted percentage of fat >5 µm or $PFAT_5$ cannot exceed 0.05% of the total dispersed phase, irrespective of the final lipid concentration. Ideally, such a limit should be extended to apply to extemporaneously prepared lipid injectable emulsion-containing TPN admixtures during their period of clinical use (28). Of note, the same technique in Method II of <729> has also served as the reference method for USP <788> entitled "Particulate Matter in Injections" (29) for more than 20 years.

Table II illustrates the ability of LE/SPOS to routinely measure this population in a variety of commercially available and stable lipid injectable emulsions of varying oil composition (26). By comparison, Table III depicts the 'stable population' of large-diameter fat globules when lipid injectable emulsions are admixed as TNA dosage forms (28). Thus, it is evident that the method is capable of measuring very small amounts of fat globules >5 µm in stable emulsion formulations. As human capillaries have an internal diameter between 4 and 9 µm, USP <729> also specifies a physiologically relevant dimension of 5 µm, a size where such globules may begin occlusion of the microvasculature. Destabilization of the lipid injectable emulsion will result in an increasing population of the large-diameter tail of the GSD, through coalescence, recognizing that changes detected in the large-diameter tail ($PFAT_5$) will have virtually no measurable effect on the MDS (14), until very late in the destabilization process (i.e., when obvious phase separation occurs with free oil that is easily detectable by the naked eye). Hence, Method II is the stability-indicating measurement indicated in <729> for lipid injectable emulsions.

PHARMACEUTICAL EXAMPLES OF APPLICATION OF SINGLE-PARTICLE COUNTING/SIZING TO LIPID INJECTABLE EMULSIONS

Most would agree that a stability-indicating method for determining the pharmaceutical integrity of lipid injectable emulsion requires quantification (i.e., single-particle or globule counting) of the large-diameter fat globules that have formed as a result of coalescence. There are basically three ways to accomplish this task, including, microscopy, electrical resistive pore method or electrical zone sensing, and the optical

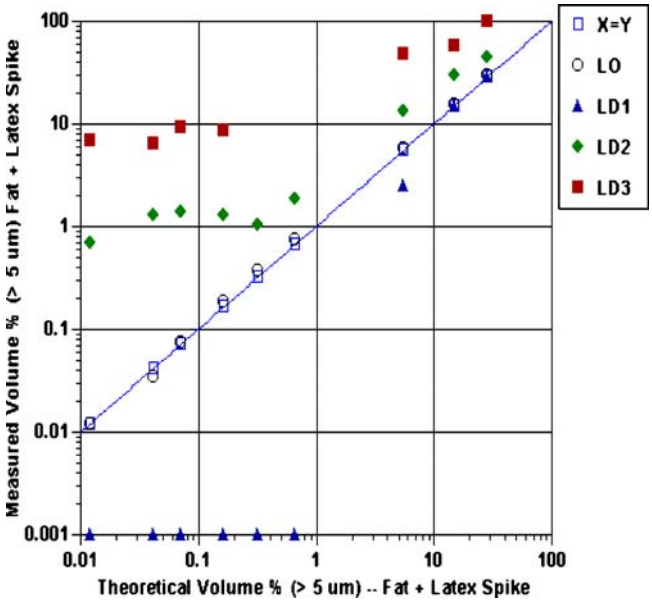

Fig. 2. Light obscuration (*LO*) three different laser diffraction instruments (*LD1*, *LD2*, *LD3*) *versus* ideal correlation (*X* = *Y*) of varying concentrations of 5-µm calibrator spheres in lipid injectable emulsions*.
*Adapted from reference 27.

Table II. Physical Characteristics of Commercially Available Lipid Injectable Emulsions

Product	Lot No.	Months to ED	GN 1.8	GN 5	GN 10	$PFAT_{1.8}$	$PFAT_5$	$PFAT_{10}$	MDS
Soybean oil only									
Intralipid 10%	12202-51	9	1224718	75148	774	0.024	**0.009**	0.0010	**286**
Intralipid 20%	10776-71	6	2983655	8645	135	0.017	**0.005**	0.0008	**340**
Intralipid 30%	16115-51	17	2017816	12504	608	0.048	**0.007**	0.0020	**420**
Liposyn III 10%	45-351-DE	18	482797	75456	5312	0.022	**0.013**	0.0040	**263**
Liposyn III 20%	43-440-DE	12	674098	73822	2320	0.010	**0.005**	0.0007	**307**
Liposyn III 30%	41-395-DE	10	2184390	340158	40984	0.040	**0.029**	0.0160	**301**
Lipofundin-N 10%	8085A83	15	321923	3856	175	0.011	**0.001**	0.0005	**272**
Lipofundin-N 20%	8082A84	15	2525720	67508	3978	0.016	**0.005**	0.0020	**332**
Soybean oil mixtures									
Liposyn II 20%	47-412-DE	16	744869	45637	1893	0.009	**0.004**	0.0010	**278**
ClinOleic 20%	9801376	16	701530	11598	785	0.004	**0.001**	0.0005	**276**
Structolipid 20%	18417-51	5	1222491	123661	4773	0.018	**0.009**	0.0020	**276**
Lipoplus 20%	9235A32	15	1816737	83642	5927	0.019	**0.008**	0.0040	**263**
Lipofundin MCT 10%	8042A81	13	438757	44930	2731	0.014	**0.008**	0.0030	**266**
Lipofundin MCT 20%	8075A81	15	1230490	114299	5708	0.016	**0.009**	0.0030	**287**
Lipovenous MCT 20%	KK1569	20	530475	15483	109	0.004	**0.001**	0.0005	**275**
Critilip 20%	KV1249B	17	9548816	205183	3723	0.051	**0.012**	0.0020	**330**

Table adapted and expanded from data in (26) and from (27).
Months to ED Months to expiration date at time of test, *GN* globule number per milliliter, *PFAT* percentage (volume-weighted) of fat determined by LE/SPOS, *MDS* mean droplet size (intensity-weighted) in nanometers determined by DLS.

equivalent of this technique, known as light obscuration or extinction. They are listed above in order of increasing statistical relevance with respect to the typical number of fat globules that can be counted and sized in practice from a given emulsion sample. It should be noted, for example, that in a stable native lipid injectable emulsion containing 300-nm droplets, an estimated 10^{12} globules/ml can be calculated to be present in the emulsion, knowing the density of oil, the mass or concentration of lipids and the volume of a sphere. As the emulsion is not a monodisperse formulation, a range of droplet sizes spanning a wide range of sphere volumes, is present, and thus the 'total' number of droplets per milliliter is not easily calculated nor determined. For clinically important large-diameter fat globules, Tables II and III show the number of globules per ml in stable lipid injectable emulsions (i.e., $PFAT_5$ <0.05%), and above 5 μm, there are approximately between 10^4 and 10^5/ml. Hence, even in this 'remote' population of the large-diameter tail of the distribution of droplet sizes, these are

Table III. Globule Size Distribution Data (Mean ± SD, $n = 6$ replicates per time interval) for TNAs Studied

Formula (kg)	Time	$GN_{1.8}$	GN_5	GN_{10}	$PFAT_{1.8}$	$PFAT_5$	$PFAT_{10}$	MDS
40	0	249252 ± 37125	39336 ± 2215	1951 ± 225	0.042 ± 0.004	**0.028 ± 0.002**	0.008 ± 0.001	**269.8 ± 0.8**
	6	226761 ± 32990	30756 ± 1109	928 ± 130	0.032 ± 0.003	**0.019 ± 0.001**	0.004 ± 0.001	
	24	165409 ± 21836	16880 ± 2149	377 ± 56	0.019 ± 0.002	**0.010 ± 0.001**	0.001 ± 0.001	
	30	162701 ± 29225	14477 ± 1870	348 ± 115	0.017 ± 0.003	**0.008 ± 0.001**	0.001 ± 0.000	**269.7 ± 0.8**
50	0	270482 ± 1193	45761 ± 1136	2194 ± 230	0.047 ± 0.002	**0.031 ± 0.002**	0.009 ± 0.001	**270.3 ± 2.9**
	6	248338 ± 3344	37056 ± 1299	1131 ± 55	0.037 ± 0.001	**0.022 ± 0.001**	0.005 ± 0.001	
	24	178941 ± 4219	20660 ± 2390	487 ± 88	0.022 ± 0.001	**0.011 ± 0.001**	0.002 ± 0.000	
	30	178152 ± 2816	17734 ± 2702	336 ± 50	0.020 ± 0.002	**0.010 ± 0.001**	0.002 ± 0.001	**269.8 ± 2.8**
60	0	316026 ± 95383	44800 ± 3268	2132 ± 152	0.048 ± 0.007	**0.030 ± 0.002**	0.009 ± 0.001	**274.4 ± 2.2**
	6	278232 ± 82985	29205 ± 1199	624 ± 53	0.032 ± 0.004	**0.016 ± 0.001**	0.002 ± 0.001	
	24	220450 ± 79561	17148 ± 878	338 ± 20	0.021 ± 0.004	**0.009 ± 0.001**	0.001 ± 0.001	
	30	210269 ± 76292	14263 ± 867	261 ± 20	0.019 ± 0.004	**0.007 ± 0.001**	0.001 ± 0.001	**270.3 ± 4.7**
70	0	256674 ± 3493	41901 ± 1408	1776 ± 167	0.040 ± 0.002	**0.026 ± 0.001**	0.007 ± 0.001	**271.0 ± 5.2**
	6	235143 ± 4595	31939 ± 1662	745 ± 137	0.030 ± 0.001	**0.017 ± 0.001**	0.003 ± 0.001	
	24	175895 ± 2132	17134 ± 1387	326 ± 27	0.018 ± 0.001	**0.009 ± 0.001**	0.001 ± 0.000	
	30	170892 ± 5603	14803 ± 1967	270 ± 65	0.017 ± 0.001	**0.008 ± 0.001**	0.001 ± 0.000	**271.7 ± 3.3**
80	0	325270 ± 76852	52727 ± 3556	3397 ± 235	0.056 ± 0.006	**0.039 ± 0.002**	0.015 ± 0.001	**271.9 ± 1.6**
	6	300842 ± 71644	39817 ± 1859	1245 ± 96	0.039 ± 0.005	**0.023 ± 0.001**	0.005 ± 0.001	
	24	229026 ± 62919	21429 ± 913	485 ± 41	0.023 ± 0.003	**0.011 ± 0.001**	0.002 ± 0.000	
	30	219017 ± 56038	18285 ± 1053	343 ± 47	0.021 ± 0.002	**0.009 ± 0.001**	0.001 ± 0.000	**270.8 ± 2.1**

Table adapted from (28).
GN Globule number per milliliter greater than 1.8, 5 or 10 um, *PFAT* percent fat (volume-weighted) greater than 1.8, 5 or 10 um (boldface values *vs.* USP <729> for $PFAT_5$ < 0.05%), *MDS* mean droplet size in nanometers (boldface values *vs.* USP <729> for lipid injectable emulsion of <500 nm).

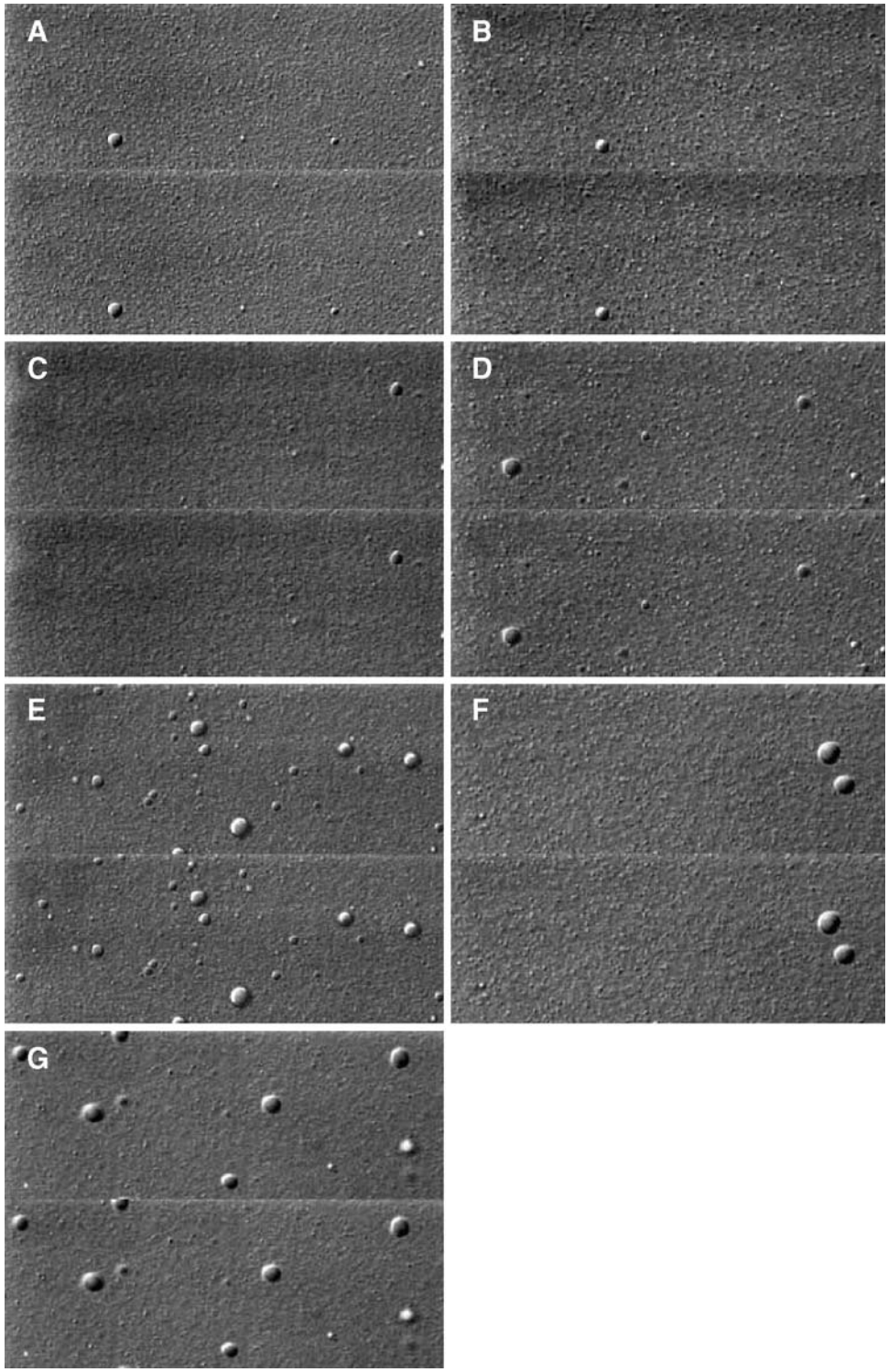

Fig. 3. (A – G)* Typical microscopic depictions for the seven $PFAT_5$ levels identified in this study using the criteria outlined in Fig. 1. The depictions chosen were intended to illustrate the progressive coarsening of the emulsion as $PFAT_5$ increases. (A) Typical microscopic depiction of $PFAT_5$ of <0.010%. (B) Typical microscopic depiction of $PFAT_5$ of <0.025%. (C) Typical microscopic depiction of $PFAT_5$ of <0.050%. (D) Typical microscopic depiction of $PFAT_5$ of <0.100%. (E) Typical microscopic depiction of $PFAT_5$ of <0.200%. (F) Typical microscopic depiction of $PFAT_5$ of <0.300%. (G) Typical microscopic depiction of $PFAT_5$ of <0.600%.
*From reference 30.

concentrated dispersions which pose significant technical and statistical issues with respect to measurements, and the ultimate determination of stable *vs* unstable lipid injectable emulsions.

Microscopic Assessments

The use of microscopy has been commonly employed over the years and can give a relative 'picture' of the stability of an emulsion. A procedure applying differential interference contrast (DIC) microscopy using an oil immersion technique has been recently described for use in lipid injectable emulsion analyses (30). The major shortcoming of microscopic techniques is the poor statistics obtained for a given measurement. The sample analyzed includes one drop of the emulsion and multiple field analyses. The number of fat globules detected microscopically in a stable formulation ($PFAT_5 < 0.05\%$) larger than 5 μm was invariably less than three fat globules per sample, while for very coarse emulsions ($PFAT_5 > 0.40\%$) the total numbers per sample were less than ten fat globules (30). Figure 3A through G show typical microscopic depictions of lipid injectable emulsions studied of varying stability and the associated measured $PFAT_5$ levels obtained via application of Method II of proposed USP <729>. Despite the statistical shortcomings, microscopy is a useful quality assurance tool for confirming the results obtained by more sensitive techniques.

Electrical Zone Sensing

Electrical zone sensing (EZS) techniques have also been used to quantify changes in the large-diameter tail of the GSD of lipid injectable emulsions. In 1992, Washington and Sizer compared the results of lipid injectable emulsion stability (as TNAs) ascertained from EZS and results obtained from laser diffraction technique over 180 days. (31) The EZS technique was determined to be superior to laser diffraction in detecting large-diameter fat globules associated with instability. Once again, as found with LE/SPOS *vs* laser diffraction (25), single-particle counting methods proved superior. The EZS data indicated "a gradual and continuous coalescence leading to large droplets" (31), with initial droplet (>1.2 μm) counts of less than 1,000 up to approximately 5,500 at 90 days, because at 180 days "mixtures showed so much aggregation that it was not meaningful to measure them." (31) Although better statistics were obtained compared to microscopy, they were still inadequate compared to those subsequently obtained using Method II of USP <729>. Moreover, there are technical concerns related to the EZS procedure in preparing the emulsion sample for analysis, and this is also evident from the aforementioned study (31). For example, with EZS assessments, "a 2 ml sample of emulsion was diluted to 200 ml in filtered water and 0.2 ml of this sample was added to 150 ml of electrolyte in the counting vessel. A 2 mL sample was drawn through the pinhole for counting." (31) Notwithstanding the need for a destabilizing supporting electrolyte to provide conductivity required for the measurement, the final sample required a pre-dilution of 75,000:1 for EZS analysis. The number of globules per ml >1.8 μm in native lipid injectable emulsions and parenteral nutrition admixtures are approximately ten times higher than those >5 μm under stable conditions, as seen in Tables II and III. The initial sizing of approximately 500 fat globules via ESZ in the Time $t = 0$ samples (i.e., stable baseline) obtained by Washington and Sizer exceeds those counted by microscopy, but is still very small compared to the actual numbers of larger fat globules actually present.

Optical Zone Sensing

Light obscuration or extinction employing a single-particle optical sensing (LE/SPOS) technique has been used for years as the reference method for USP <788>, and more recently for stability assessments of lipid injectable emulsions when used as TNAs (32–34). It was not until 1995 that such quantitative assessments were correlated with visual evidence of phase separation in an attempt to "provide evidence to support its [light obscuration] application to intravenous fat emulsions and TNA formulations." (14). In this study, a series of 90 TNAs was studied using visually obvious phase separation as a standard measure, "a TNA with >0.4% of its total fat concentration present as particles of >5 μm would crack 85% of the time, whereas a TNA with <0.4% of it total fat concentration present as particles >5 μm would be stable 88% of the time." (14) Stability was defined as the absence of visually evident phase separation (e.g., large free oil globules located at the surface of a standing emulsion or adhering to the walls of the infusion container). Clearly, phase separation is a terminal event in the destabilization of emulsions, which assumes particular clinical importance if given by intravenous infusion. Unfortunately, as the above study noted "...instability was visibly evident only 65% of the time" (14), which demonstrated the need for heightened instrumental surveillance of injectable pharmaceutical emulsions.

Since 1995, several studies employing LE/SPOS have been published that have differentiated between stable and unstable lipid injectable emulsions as TNAs. These have shown differences in stability based on, for example, effects from in-line filtration (35), adult TNAs (36), the composition of the oil phase (37), specialized infant TNAs (15), compounding techniques (16), and in extreme patient conditions (28). In 2001, a study which validated the LE/SPOS technique, also reported the current globule size profiles of 16 different commercially available native lipid injectable emulsions employing the currently proposed Method I (light-scattering) and Method II (LE/SPOS) of USP <729> (25). It concluded with the following pharmacopeial recommendation: "For commercial IVLE [intravenous lipid emulsions] 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% (25)." Furthermore, it stated: "...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 (25)." Of the 16 emulsions studied, the $PFAT_5$ values of six U.S products (Intralipid® and Liposyn®, 10, 20 and 30% each) are depicted in Fig. 4 as log $PFAT_5$ *vs* lipid concentration, showing all these emulsions to be within the recommended $PFAT_5$ limit of <0.05%.

In 2004, there was a significant change in the packaging of lipid injectable emulsions resulting in the introduction of a

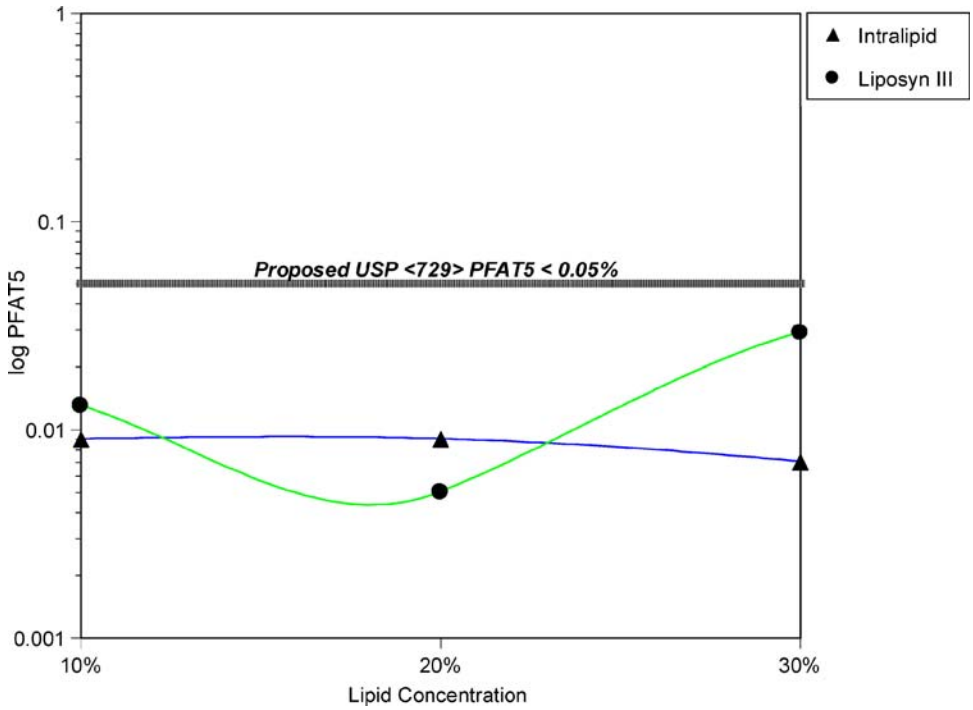

Fig. 4. Large-diameter profile of US-based lipids packaged in conventional glass bottles*.
*Adapted from reference 25; cubic spline curve fit.

plastic container to replace the conventional glass container for one of the US products. A subsequent analysis of these new lipid injectable emulsion dosage forms showed significant coarsening of the emulsion and failure in every case to meet the proposed USP <729> limits of Method II (i.e., $PFAT_5 < 0.05\%$) (38), whereas in its glass counterpart, the product passed. A comparison of these results is plotted in Fig. 5 for four separate lots of plastic- *vs* glass-based 20% lipid injectable emulsions showing the differences between formulations. Subsequently, in a study investigating the

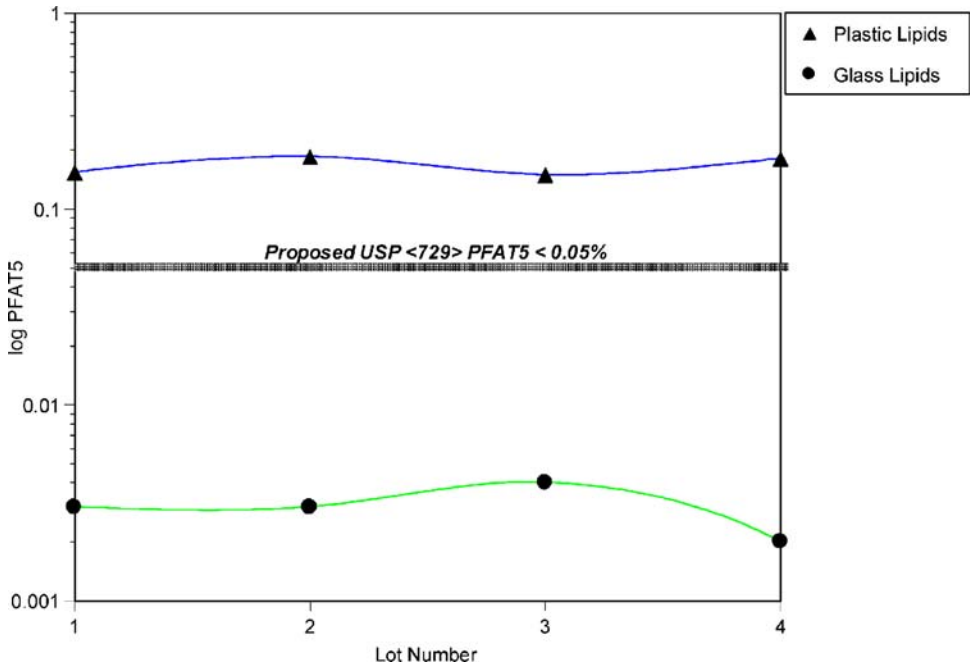

Fig. 5. Large-diameter profile of US-based lipids packaged in plastic containers versus conventional glass bottles*.
*Adapted from reference 38; cubic spline curve fit.

implications of the changes in the plastic-based lipid injectable emulsions, a comparative stability analysis was performed to assess whether the coarsened GSD found in plastic containers resulted in less stable TNAs than those made from conventional glass-based lipids (39). Fifteen TNAs designed for adult patients were tested for each lipid, and 60% of the admixtures made from plastic had high PFAT profiles (i.e., $PFAT_5 > 0.05\%$) and cracked within 30 h, while none of the glass-based TNA formulations was unstable by either criterion. Thus, it appears that the present plastic-based lipid injectable emulsions do not pass the proposed USP <729> large-diameter globule size limits, and also produce less stable TNAs than those made from conventional glass bottles.

PHYSIOLOGICAL SIGNIFICANCE OF INFUSING UNSTABLE LIPID INJECTABLE EMULSIONS

The conventional view of clinical harm in association with the intravascular infusion of an unstable, large fat globule-laden lipid injectable emulsion is the development of a 'fat overload syndrome'. In this case, metabolic clearance is greatly impaired with possible obstruction of the microvasculature, causing, for example, capillary fat embolism. In a classic review of the status of parenteral nutrition published in 1960 by Robert Geyer (40), a pioneer in the development and study of lipid injectable emulsions in the USA, the clinical problems encountered with the infusions appeared in two forms. First, acute adverse effects included pyrogenic reactions also known as the 'colloid reaction' and occurred within 10–20 min of an infusion. They were accompanied by chest or back pain, cyanosis, flushing and dyspnea, characteristic signs and symptoms of an anaphylactoid reaction. Second, sub-acute reactions, also known as 'fat overload syndrome', were observed to occur after several days of infusion and were associated with bleeding tendency, fever, epigastric pain, jaundice and "accumulation of sudanophilic material in sinusoidal macrophages of the liver" (40). In fact, Geyer further states "A brownish pigment–lipoid complex termed intravenous fat pigment has been found in the Kûpfer cells of the liver and reticuloendothelial cells of the spleen of humans and animals after fat emulsion administration" (40).

The *acute reaction* reported during the early use of lipid injectable emulsions seems to have subsided with current experience, and is most likely from improved manufacturing techniques. In fact, Geyer suggested the early problems were indeed manufacturing-related when he stated the following: "The fact that a given patient can react to one batch of emulsion but not necessarily to another of the same composition and ingredients, cannot be overlooked" (40). Nonetheless, the FDA-approved package inserts of US lipid injectable emulsions do recommend the use of (but not widely practiced) a 'test dose' of lipids of 0.5 ml/min for up to 30 min, which can then be increased if no untoward reactions occur. On the other hand, the *sub-acute reactions* that are associated with fat overload are still relevant today, as it can be induced by excessively high infusion rates and/or when given to patients with pre-existing impaired plasma clearance mechanisms. In fact, the FDA-approved package inserts for US lipid injectable emulsions contain a Black Box Warning for premature infants and neonates, reproduced in Table IV.

So clearly, the risk of fat overload syndrome from impaired clearance of infused lipids is a major concern of the FDA. Increasing the intravascular concentration of large-diameter fat globules, a consequence of droplet coalescence occurring in unstable lipid injectable emulsions, will also alter their normal metabolic fate. Under optimal physiological conditions, submicron lipid droplets are systematically metabolized by lipoprotein lipase found along the vascular endothelium. In contrast, infusions of large fat globules (>1 μm) are rapidly cleared, and although their binding sites and destinations are not known (41), their metabolic fate is most likely determined by phagocytosis via macrophages of the reticuloendothelial system (RES). The RES is an important component of the immune defense system representing fixed macrophages lining the sinusoids and microvasculature of tissues of the lungs, liver, bone marrow and spleen, and therefore the finding of lipids in these organs would be consistent with Geyer's earlier observations. Consequently, the macrophages will phagocytize xenobiotics, such as large-diameter fat globules, producing reactive oxygen species (ROS). The byproducts of polyunsaturated fatty acid peroxidation, such as ROS, can be assessed by measuring the amount of malondialdehyde (MDA) present in these tissues. Therefore, in less stable lipid injectable emulsions, i.e., those with a higher proportion of large-diameter fat globules (i.e., >5 μm, reported as $PFAT_5$ by USP <729>), it would be expected that an increasing concentration of MDA would be found in affected tissues, such as the lungs and liver. Therefore, in order to establish a dose-related toxicity (or LD_{50}), it is necessary to employ a quantitative method to identify large fat globule doses, such as described in Method II of USP <729>.

TOXICOLOGICAL MODELS

In two separate animal models using MDA as a 'surveillance marker' of oxidative stress, we have shown significant increases in this chemical component in both the lung and liver tissues. In a guinea pig model (42), the lung tissue concentrations of MDA were significantly higher in the group

Table IV. Lipid Injectable Emulsion Black Box Warning from the Manufacturer's Product Package Insert

Deaths in premature infants after infusion of intravenous fat emulsion have been reported in the medical literature. Autopsy findings included intravascular fat accumulation in the lungs. Treatment of premature and low birth weight infants with intravenous fat emulsion must be based upon careful benefit–risk assessment. Strict adherence to the recommended total daily dose is mandatory; hourly infusion rate should be as slow as possible in each case and should not in any case exceed 1 g fat/kg in 4 h. Premature and small for gestational age infants have poor clearance of intravenous fat emulsion and increased free fatty acid plasma levels following fat emulsion infusion; therefore, serious consideration must be given to administration of less than the maximum recommended doses in these patients in order to decrease the likelihood of intravenous fat overload. The infant's ability to eliminate the infused fat from the circulation must be carefully monitored (such as serum triglycerides and/or plasma free fatty acid levels). The lipemia must clear between daily infusions.

From (10) and (11).

receiving a very unstable (almost 50-fold higher than the proposed USP <729> limit of $PFAT_5$ < 0.05%) lipid injectable emulsion (average $PFAT_5$ = 2.42%) *vs* a stable one (average $PFAT_5$ = 0.004%). No differences in liver tissue concentrations were noted. In two subsequent rat studies of similar infusions over 24 (unstable average $PFAT_5$ = 0.682% or 13.6x USP <729> limits) or 72 h (unstable average $PFAT_5$ = 0.117% or 2.3x USP <729> limits) (43), significantly higher amounts of MDA were found in liver tissues of animals receiving the unstable lipid *vs* those receiving stable lipid emulsions ($PFAT_5$ <0.05%). The difference in organs affected between species was most likely related to the degree of instability, with the highest $PFAT_5$ associated with significant accumulation in the lungs, which is the principal site of exposure upon intravascular infusion. Hence, significant retention of large fat globules would accumulate in the lungs, as shown in the guinea pig study. At lower $PFAT_5$ concentrations, the liver appears to be the main organ affected, as seen in the rat studies. In addition to oxidative stress observed in the livers of rats, the elevated MDA levels were also associated with significantly higher AST concentrations in plasma, suggesting hepatic injury.

The toxicity associated with the infusion of unstable lipid injectable emulsions is principally related to impairments in plasma clearance of the infusion. On a 'macroscopic' scale, when this occurs, serum triglycerides and free fatty acids may be elevated, which may be associated with significant morbidity and mortality in critically ill premature infants, neonates and newborns as per the Black Box Warnings in the package insert. On a 'microscopic' scale, the clearance of large fat globules is greatly accelerated, and they most likely accumulate in RES organs where they can cause increased oxidative stress and possible organ injury. With respect to the role of the RES in the clearance of lipid injectable emulsions, previous animal data (43,44) and human data (45,46) would suggest that the liver is the principal organ injured by the infusion of unstable lipid injectable emulsions, as originally described by Geyer (40).

CLINICAL PRESENTATION OF TOXICITY FROM LIPID INJECTABLE EMULSIONS

The toxicity likely will manifest in two possible clinical conditions. In the first condition, the acute development of toxicity from either the infusion of excess doses of lipid or from gross destabilization of the lipid injectable emulsion will most likely exhibit overt clinical signs and symptoms. A clinical case report that best illustrates this condition comes from a case study involving a 31-year-old pregnant patient who developed *hyperemesis gravidarum* at 7-weeks' gestation (47). Over the ensuing 5–6 weeks, the patient lost 20 lb, and TPN with lipid emulsion was initiated at 13-weeks' gestation and she subsequently regained her weight. Then, "On routine prenatal examination at 22-weeks' gestation, ultrasonography failed to detect fetal heart activity. Labor was induced with oxytocin, prostaglandin suppositories and laminaria, and she subsequently delivered a 540-g (68th percentile for gestational age) stillborn male fetus" (47). Pathological examination of the placenta with Oil red O staining showed fat globules in the syncytial cells and Hofbauer (macrophage) cells of the chorionic villi and the decidual cells, as well as numerous membrane bound, uniformly dense vacuoles with no internal structure (characteristic of lipid droplets) found via electron microscopy. These findings, occurring within nine weeks of beginning TPN with lipid injectable emulsion, strongly implicated an exogenous source of embolic fat globules that played a role in the intrauterine death.

In other cases, the clinical presentation of unstable lipid injectable emulsions may be less obvious, but they may nevertheless cause significant clinical harm in susceptible patients. For example, premature infants and neonates who are critically ill, such as indicated in the Black Box Warning of both available US lipid injectable emulsions, have poor plasma clearance and increased lipid levels, and possibly lead to 'intravenous fat overload'. In some cases, this condition has proven to be fatal.

CONCLUSIONS

The administration of lipid injectable emulsions has spanned more than 40 years of experience in patients worldwide requiring parenteral nutrition support. During this time, much has been learned in the experimental and clinical settings, despite the absence of formal pharmacopeial specifications. The USP is poised to adopt chapter <729> and the accompanying Lipid Injectable Emulsion monograph, after several failed attempts to do so going back 15 years. Of the two methods proposed in USP <729>, Method II or LE/SPOS is the stability-indicating test which will ultimately determine the safety of these complex formulations by focusing on the large-diameter fat globules arising from instability of the dispersion.

Moreover, the application of LE/SPOS has also been used to assess the toxicity of unstable lipid injectable emulsions in the experimental setting. Specific evidence in rat infusion models have linked $PFAT_5$ values with evidence of increased oxidative stress (i.e., ↑ (MDA]) and injury (i.e., ↑ [AST]) on liver tissues. In one case, a $PFAT_5$ value of 0.5–0.7% at the beginning of the infusion produced hepatic damage within 24 h, whereas in another case, a modestly unstable starting $PFAT_5$ infusion of approximately 0.1% produced the same adverse effects within 3 days, suggesting a cumulative toxic effect. These results were significantly different for the animals receiving stable lipid injectable emulsion ($PFAT_5$ <0.05%) in both infusion studies (43). Hence, from a pharmaceutical standpoint, the LE/SPOS method can discern stable from unstable lipid injectable emulsions. As well, from a toxicological perspective, LE/SPOS can identify $PFAT_5$ levels capable of producing organ damage in experimental animals.

Clearly, more experimental work is needed to corroborate the pathophysiological effects in animals and potentially in humans from infusion of unstable lipid injectable emulsions. Nonetheless, adoption of the proposed USP <729> and accompanying Lipid Injectable Emulsion monograph at this time appears appropriate.

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