The effect of structured triglycerides on the kinetic stability of total nutrient admixtures

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ABSTRACT Purpose The physical stability of two types of total parenteral nutrient (TPN) admixtures was studied as a function of storage time and temperature. One of them contained only structured triglycerides and the other exclusively long-chain triglycerides as lipid components. **Methods** Droplet size of the mixtures was followed by photon correlation spectroscopy for 10 days. Zeta potential and dynamic surface tension measurements were carried out to evaluate the possible changes in the charge and interfacial surface tension of the emulsion droplets during the storage. pH values were monitored in order to follow the possible decomposition processes in the course of storage. **Results** Droplet size of emulsions prepared with lipids containing exclusively long-chain triglycerides showed remarkable increase after 4 days of storage in contrast with that of the mixtures containing structured lipids. **Conclusions** The obtained results indicate that besides the advantageous metabolic effects of structured triglycerides, their application is recommended to improve the physical stability of TPN admixtures.

INTRODUCTION

Pharmaceutical-grade intravenous lipid emulsions are complex dispersions of oil droplets that have been carefully homogenized to produce high-quality dispersions, safe for intravenous administration, with particles of a mean dimension approximately 300 nm in diameter. This mean lipid droplet size is within the typical range of the dimensions of endogenous chylomicrons (80 – 500 nm), and the formulations are manufactured in this way to behave in a similar manner with respect to their metabolic fate (1, 2).

Intravenous lipid emulsions are systems of high physicochemical stability, thus their shelf life can be as long as 24 months when stored at 25°C. Washington et al. investigated the stability of Intralipid and Ivelip infusions, and found that the emulsions could be considered stable even after being subjected to accelerated tests such as autoclaving (3). As total nutrient admixtures are solutions comprising 60 or more chemical species in a single container, destabilization of lipid emulsions often occurs, which results in reversible aggregation or flocculation of the droplets, followed by irreversible coalescence after relatively short storage intervals (4, 5). When the volume-weighted percentage of fat at a threshold of five μm exceeds 0.4% of the total lipids present, danger of fat embolism reaches a critical level (6).

Several methods can be used for the assessment of physical stability of lipid emulsions, including particle size analysis via photon correlation spectroscopy, light obscuration, laser diffraction or microscopy (7-9). While these methods can follow physical changes, zeta-potential and pH measurements are able to indicate chemical processes that take place along with storage. Dynamic surface tension measurements can provide additional information concerning the physicochemical processes that take place on the surface of the lipid droplets.

Electrolytes added to the mixtures affect the stability of emulsions via alteration of the zeta-potential caused by the negatively charged head groups of phospholipids used as emulsifying agents in most parenteral lipid emulsions (4). It has been shown in previous works available in the literature that the type of triglycerides in the lipid component also influences the stability of all-in-one mixtures. It has been reported that pure long-chain triglyceride (LCT)-based admixtures degrade to a much greater extent than those containing medium-chain triglycerides (MCTs) and LCTs. However, the stabilizing effect of MCTs is lost when physical mixtures of MCTs and LCTs are made extemporaneously from two separate starting emulsions (10, 11).

Structured triglycerides (STs), in which both medium-chain fatty acids and long-chain fatty acids are esterified to the same glycerol molecule, have positive metabolic effects, which make them competitive or even more efficient as an energy

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source compared with conventional fat emulsions (12). They are assumed to provide a higher oxidation rate, faster clearance from blood, improved nitrogen sparing, and less of a tendency to accumulate in the reticuloendothelial system compared with LCT emulsions (13, 14).

Although the advantageous metabolic effects of STs have been widely studied, their impact on the physicochemical properties of TPN mixtures has not been clarified yet. The purpose of the present work was to study the effect of STs on the kinetic stability of total parenteral admixtures in comparison with lipids containing exclusively LCTs.

MATERIALS AND METHODS

Table 1 summarizes the composition and Table 2 comprises the total ionic concentrations of the prepared TPN (Total Parenteral Nutrition) mixtures.

Table 1 Composition of the TPN mixtures

Compounds	Quantity (ml)	
	TPN mixture 1	**TPN mixture 2**
Infusio glucosi 40% (University Pharmacy of the Semmelweis University, Budapest) Glucose anhydrate 400 g Hydrochloric acid 0,1N 1,000 ml per 1000 ml solution	500	500
Elektrolit A (University Pharmacy of the Semmelweis University, Budapest) Sodium chloride 4.675 g Potassium chloride 3.727 g Magnesium sulfate cryst 2.00 g Aqua destillata pro inj. ad 100.0 ml	100	100
Aminoven 10% 500ml inf. (Fresenius Kabi AB Sweden) L-isoleucine 5.00 g, L-leucine 7.40 g, L-methionine 4.30 g, L-lysine-acetate 9.31 g (=6.6 g L-lysine), L-phenylalanine 5.10 g, L-threonine 4.4 g, L- tryptophane 2.00 g, L-valine 6.20 g, L-arginine 12.0g, L-hystidine 3.00 g, L-alanine 14.0 g, Glycine 11.0 g, L-proline 11.2 g, L-serine 6.50 g, L-tyrosine 0.40 g, Taurine 1.00 g per 1000 ml solution Total amino acid content 100.0 g/l	1000	1000
Intralipid 20% inf. (Fresenius Kabi, Germany GmbH) Soybean oil: 200 g Purified egg phospholipids: 12 g Glycerol (anhydrous) (Ph Eur): 22.0 g Water for injection to 1000 ml	500	-
Structolipid 20% inf. (Fresenius Kabi, Germany GmbH) Structured triglycerides: 200 g Purified egg phospholipids: 12 g Glycerol (anhydrous) (Ph Eur): 22.0 g Water for injection to 1000 ml	-	500

Table 2 Ionic concentrations of the prepared TPN mixtures

Compounds	Concentration (mol/dm^3) in the TPN mixture
Na^+	0.0380
K^+	0.0238
Mg^{2+}	0.0039
Cl^-	0.0618
SO_4^{2-}	0.0039

Preparation of the TPN Mixtures

The blending of the compounds of various TPN systems was carried out in a laminar airflow box (Relatec, Germany) under vacuum. The final preparations consisted of four different types of basic ingredients: amino-acids, carbohydrates, electrolytes and lipids. The blending of the compounds was carried out under vacuum in a completely closed system. First, half of the volume of the Glucose inf. was sucked into the plastic bag through one of the plastic tubes that was connected to the bag. The electrolytes were added to the remained volume of Glucose infusion and then sucked into the plastic bag. Next, amino-acids were blended to the obtained solution. The last step was the addition of lipids to the solution by sucking the lipid emulsions into the plastic bag. The right order of the blending assured the homogeneity of the TPN mixtures.

Storage of the Prepared TPN Mixtures

The TPN mixtures were stored at 2-8 °C and 37 ± 0.5°C temperatures for 10 days.

Photon Correlation Spectroscopy

The particle size distribution of emulsions of two different compositions was examined before storage and after 4, 7 and 10 days. Dynamic light scattering measurements were carried out for checking the kinetic stability of the TPN emulsions. The apparatus (Brookhaven Instruments Corporation) used consisted of a BI-200SM goniometer and a BI-9000BO Correlator. An Argon-Ion Laser (Omnichrome 543 AP) set to the wavelength of 488 nm was applied as a light source. The homodyne autocorrelation function in channel 238 was determined at real time mode using logarithmic timescale with a range of 1-200000 μs. Detector angle was set to 90.0 deg., and the gap was 100 μm. Before the measurements, the emulsions were diluted to reach the appropriate count rate value. The time of measurement was 180s. Six parallel examinations were carried out on each sample (four different samples – according to the temperature of storage and the type of lipid emulsion used for the preparation). Data were evaluated assuming an exponential distribution of the emulsion particles. The results were plotted as intensity vs. particle size of the emulsion droplets.

Zeta-potential Measurements

Laser Doppler-electrophoresis (LDE) was used for investigating the surface-electric properties of the emulsion droplets. Measurements were carried out before storage and after 4, 7 and 10 days. For electrically charged particles moving in response to an applied electric field, a correlation function of laser Doppler-shift was measured with a Malvern Zetasizer 4 apparatus at 25 ± 1°C (Malvern Instruments, UK), and the resulting frequency spectrum was translated to electrophoretic mobility. Using an AZ 104 type cell, 5 mobility measurements were ordinarily done on each sample (four different samples – according to the temperature of storage and the type of lipid emulsion used for the preparation) in cross beam mode. The zeta potential (ζ) of the particles was calculated from the mobility measurements, using the Smoluchowsky formula.

pH Measurements

pH values of the TPN mixtures were measured right after preparation and after 1, 4, 7 and 10 days of storage with a Radelkis OP-300 electroanalytical analyser.

Dynamic Surface Tension Measurements

The examinations were carried out on the day of preparation and after 1, 4, 7 and 10 days. The surface tension of emulsions was determined by dynamic method, applying Du-Noüy ring and Wilhelmy plate operations of a computer-controlled KSV Sigma 70 tensiometer (KSV Sigma 70, RBM-R. Braumann GmbH, Germany) at 25°C ± 0.5 °C. The method determines the maximum mass of liquid pulled from the surface by lifting the specified solid (e.g. ring or plate). The force (f) measured on the electric balance is necessary for lifting out and pushing down the solid measuring device from the surface of the liquid. The contact angle can be calculated from the extrapolated buoyancy slope:

$$\cos\theta = f/p\gamma_{LV} \qquad (1)$$

where θ is the contact angle, f is the force measured on the balance, p is the measured plate perimeter and γ_{LV} is the surface tension (interfacial free energy between the liquid and vapour) of the examined liquid. 3 parallel measurements were carried out on all four kinds of samples.

Statistical Evaluation

Zeta-potential values of the two kinds of mixtures at different temperatures and storage intervals were compared using the two-sample t-test assuming equal variances. In this case, the comparison was made between Intralipid-containing infusions and

Structolipid-containing ones. Surface tension values measured after different storage intervals were compared via the paired two-sample t-test for both kinds of mixtures. The comparison was made between data obtained right after preparation and after 1, 4, 7 and 10 days, respectively.

The statistics were calculated using Microsoft Excel 2002.

RESULTS AND DISCUSSION

Figures 1-2 illustrate the average droplet size of the two different TPN emulsions at different storage temperatures. The mean droplet size of Structolipid 20% before mixing with the other components was reported to be 276 nm (9) and proved to be between 300-400 nm in the admixtures at zero time. The results unambiguously indicate that the average droplet size of emulsions containing structured triglycerides did not significantly change during the examined storage period. In contrast, the droplet size of emulsions prepared with lipids containing exclusively long-chain triglycerides, showed remarkable increase even after 4 days of storage. The mean droplet size of Structolipid 20% before mixing with the other components was reported to be 276 nm (9) and proved to be between 300-400 nm in the admixtures at zero time. As commercially available lipid emulsions can be stored for 24 months, these findings confirm the fact that the additives mixed to these systems negatively influence their stability.

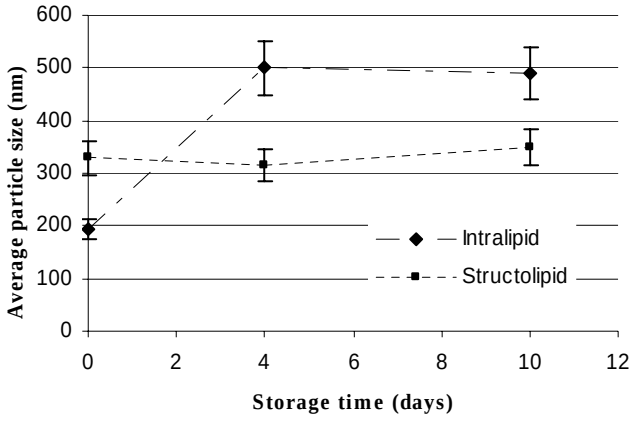

Figure 1 – Effect of storage time on the average droplet size of the prepared TPN systems; Storage temperature: 2-8 ^{0}C

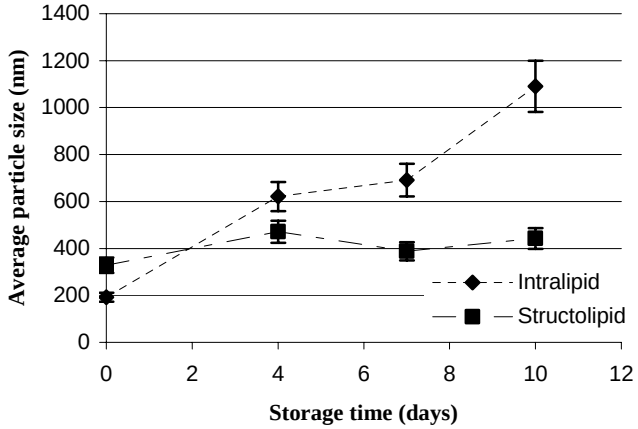

Figure 2 – Effect of storage time on the average droplet size of the prepared TPN systems; Storage temperature: 37 ± 0.5 ^{0}C

Table 3 shows the zeta-potential values of the two mixtures after storing at different temperatures for 10 days. Such values of intravenous lipid emulsions can be found in the literature and are in the range of -40 to -50 mV (4), which shows remarkable increase (i.e. weaker repulsive forces between the droplets) in the admixtures. No significant difference could be observed between the two kinds of compositions at zero time, which suggests that their initial stability can be considered equivalent. p values indicate significant differences between the two compositions after 4 and 7 days of storage. The more negative zeta-potential values of the mixture containing structured lipids confirm the results of the particle-size analysis, i.e. the enhanced stability of the system prepared with Structolipid. After 10 days, the zeta-potential values can be considered equivalent again, which is probably the result of the starting destabilization process of the composition containing structured lipids.

Since the ionic concentration of the two TPN emulsions was equal and pH values measured in the course of storage (Table 4) did not present remarkable changes, the lower physicochemical stability of emulsions prepared with LCTs can not be ascribed to electrostatic effects or chemical decomposition.

Very likely, the formation of a "mixed" interfacial layer formed from the medium and long chain fatty acids in case of structured triglycerides is responsible for the more efficient stabilization. The latter could be tracked by the different interfacial surface structure of the dispersed droplets.

Table 3 Electrokinetic characteristics of different TPN emulsions (average of 5 parallel measurements, ± S.D.; α = 0.05)

Storage time (days)	Temperature (°C±0.5°C)	Zeta potential (mV)		p
		Intralipid	Structolipid	
0	25	-2.2 ± 0.10	-1.9 ± 0.35	> 0.05
4	2-8	-2.4 ± 0.15	-2.9 ± 0.15	< 0.05
4	37	-3.0 ± 0.40	-4.1 ± 0.40	< 0.05
7	2-8	-1.7 ± 0.05	-2.9 ± 0.60	< 0.05
7	37	-2.7 ± 0.60	-3.9 ± 0.15	< 0.05
10	2-8	-2.0 ± 0.20	- 2.9 ± 0.90	> 0.05
10	37	-3.3 ± 0.05	- 2.9 ± 0.40	> 0.05

Table 4 pH values of the mixtures before and after storage under different conditions (average of 3 parallels, ± S.D.)

Storage time (days)	Temperature (°C±0.5°C)	pH	
		Intralipid	Structolipid
0	25	5.8 ± 0.1	5.8 ± 0.2
1	2-8	5.7 ± 0.1	5.9 ± 0.1
1	37	5.7 ± 0.2	5.7 ± 0.1
4	2-8	5.9 ± 0.2	6.0 ± 0.1
4	37	5.8 ± 0.1	5.8 ± 0.2
7	2-8	5.9 ± 0.1	5.9 ± 0.2
7	37	5.7 ± 0.1	5.7 ± 0.3
10	2-8	5.9 ± 0.2	5.9 ± 0.1
10	37	5.7 ± 0.1	5.7 ± 0.2

The surface tension values measured by the Wilhelmy plate operations are summarized in Table 5. The measured surface tension of purified water was 58.81 ±0.113 mN/m. The surface tension values determined with Du-Noüy ring correlated well to values measured by the plate method, but the latter resulted in higher accuracy. As it can be seen in Table 5, the obtained surface tension remained almost constant within the examined storage intervals in the case of admixtures containing the structured lipid component – indicating a more stable interfacial surface structure. p values indicate significant difference compared to zero time only after 10 days of storage at 2-8°C. In contrast, the surface tension of emulsions containing exclusively long-chain triglycerides remarkably decreased during storage referring to the interfacial structural changes. In the case of the sample stored at 37°C, a significant change could be observed after 4 days. Although further studies are needed to elucidate the mechanism of the (steric) stabilization, dynamic surface tension measurements can be recommended as sensitive means for the stability tests of intravenous lipid emulsions.

Table 5 Surface tension values of different TPN emulsions stored under different conditions (average of 3 parallels, ± S.D.). p refers to the comparison of the surface tension values with the corresponding values at zero time (α = 0.05).

Storage time (days)	Surface tension (mN/m)							
	Structolipid				Intralipid			
	2-8°C	p	37°C	p	2-8°C	p	37°C	p
0 (25°C)	30.49 ±0.384	-	30.49 ±0.326	-	33.48 ±0.620	-	33.48 ±0.408	-
1	30.90 ±0.846	>0.05	30.28 ±0.846	>0.05	33.06 ±0.887	>0.05	31.53 ±0.725	<0.05
4	30.39 ±0.164	>0.05	30.47 ±0.095	>0.05	28.12 ±0.867	<0.05	24.33 ±0.826	<0.05
7	30.19 ±0.503	>0.05	30.47 ±0.437	>0.05	26.16 ±0.584	<0.05	26.36 ±0.500	<0.05
10	32.17 ±0.342	<0.05	31.50 ±0.425	>0.05	27.58 ±0.872	<0.05	27.06 ±0.537	<0.05

The findings of this study are in good correlation with the results of Driscoll et al. concerning the stability of all-in-one admixtures containing MCTs and LCTs previously mixed in a single emulsion or added separately to the mixtures (10). As it was reported, separate droplets of MCTs and LCTs resulted in impaired physicochemical stability compared to the ones containing both kinds of triglycerides. In the case of structured lipids, both medium and long chain fatty acids can be found in the starting lipid emulsion, leading to a favourable interfacial location of structured triglycerides.

The clinical significance of the present study lies in the recognition that with the application of total nutrient admixtures containing structured lipids, the incidence of fatal consequences of parenteral nutrition (e.g. fat embolism) could be decreased.

CONCLUSIONS

Kinetic stability of two total nutrient admixtures prepared with different lipid emulsions (Intralipid and Structolipid, respectively) was tracked for 10 days with an array of physicochemical methods. Besides the commonly applied techniques such as photon correlation spectroscopy and zeta-potential measurements, dynamic surface tension studies can contribute to the evaluation of the stability of TPN mixtures. In addition to the advantageous metabolic effects of structured triglycerides, their application is recommended also to improve the physical stability of TPN admixtures, which could decrease the risk of fat embolism in the clinical practice.

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