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Tracking of the Kinetic Stability of 2 Types of Total Nutrient Admixtures Containing Different Lipid Emulsions

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ABSTRACT

The physical stability of 2 types of total nutrient 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. To evaluate the possible changes in the kinetic stability of the emulsions and in the surface characteristics of the droplets during storage, particle size analysis, zeta potential, and dynamic surface tension measurements were performed. To follow any chemical decomposition processes that occurred during storage, the pH of the emulsions was also monitored. The mean droplet size of emulsions prepared with lipids containing exclusively long-chain triglycerides showed a remarkable increase after 4 days of storage, in contrast with that of the mixtures containing structured lipids. A combination of size distribution, zeta potential, and dynamic surface tension measurements proved to be useful for an adequate tracking of the kinetic stability of total nutrient admixtures. Structured triglycerides not only provide advantageous metabolic effects but improve the physical stability of total parenteral nutrition admixtures.

KEYWORDS: Nutrient admixtures, structured triglycerides, long-chain triglycerides, kinetic stability, zeta potential, dynamic surface tension.

INTRODUCTION

Lipid emulsions have been used in routine clinical practice for more than 40 years. Intralipid, which was the first well-tolerated lipid emulsion, is still the most commonly used product worldwide. It contains long-chain triglycerides (LCTs) with a fatty acid chain length of 16 to 20 carbon atoms (long-chain fatty acids).

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Suggested disadvantages of LCTs are their slow elimination from the bloodstream; their relatively high content of ω-6 polyunsaturated fatty acids, in particular linoleic acid; a relatively high rate of reesterification; and storage of triglycerides in various tissues.

It has also been suggested that LCTs may interfere with the immune system, but this has not been demonstrated in clinical practice.[1]

There is an alternative concept to conventional soybean oil emulsions with a physical mixture of medium-chain triglycerides (MCTs) and LCTs or as structured triglycerides (STs, in which both medium-chain fatty acids and long-chain fatty acids are esterified to the same glycerol molecule). MCTs, which are triglycerides composed predominantly of fatty acids with 8 or 10 carbon atoms (medium-chain fatty acids), have been reported to be metabolized faster than LCTs, with little or no storage of medium-chain fatty acids in tissues; to be oxidized partly independent of carnitine; and to entail less effect on reticuloendothelial function.[2-4] Consequently, in clinical practice, physical mixtures of MCT/LCT emulsions have been on the market since the 1980s and STs since the 1990s.

From a physicochemical point of view, 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 ~300 nm in diameter. This mean lipid droplet size is within the typical range of the dimensions of endogenous chylomicrons (80-500 nm). The formulations are manufactured so they behave as endogenous chylomicrons do with respect to their metabolic fate.[5,6]

As total nutrient admixtures are solutions comprising 60 or more chemical species in a single container, there is often destabilization of lipid emulsions, which results in reversible aggregation or flocculation of the droplets, followed by irreversible coalescence after relatively short storage intervals.[7,8] When the volume-weighted percentage of fat at a threshold of 5 μm exceeds 0.4% of the total lipids present, the danger of fat embolism reaches a critical level.[9]

The physical stability of lipid emulsions can be tracked by an array of techniques, including particle size analysis via

photon correlation spectroscopy, light obscuration, laser diffraction, or microscopy.[10-13] While these methods can follow physical changes, zeta potential and pH measurements are able to indicate chemical processes that take place during storage. Dynamic surface tension measurements can provide additional information concerning the physicochemical processes that occur on the surface of the lipid droplets.

The effect of several factors on the stability of total nutrient admixtures has been studied by several authors.[8-14] Electrolytes play an especially important role in stability, as they are present in all admixtures and have a major effect on the zeta potential of the emulsions. In the case of nonspecific adsorption, they physically adhere to the surface of the lipid droplets and above the critical flocculation concentration (CFC) cause the disappearance of repulsive forces. Specific adsorption occurs when there are both physical and chemical interactions (eg, Ca^{2+} and phospholipids). In this case, further adsorption is possible above the CFC and repulsive forces arise again.[8]

It has been shown that the type of triglycerides in the lipid component also influences the stability of all-in-one mixtures. It has been reported that pure LCT-based admixtures degrade to a much greater extent than do those containing MCTs and LCTs. However, the stabilizing effect of MCTs is lost when physical mixtures of MCTs and LCTs are made extemporaneously from 2 separate starting emulsions.[15,16] We have previously reported that besides their desirable metabolic effects, STs also contribute to the enhanced physicochemical stability of total nutrient admixtures.[17]

The purpose of the present study was to compare the kinetic stability of 2 admixtures containing different lipid components. A further aim was to collect more evidence for the stabilizing effect of STs, with special attention to the ionic concentration of the mixtures.

Materials and Methods

Table 1 summarizes the composition and Table 2 lists the total ionic concentrations of the prepared total parenteral nutrition (TPN) mixtures.

Preparation of TPN Mixtures

The blending of the compounds of various TPN systems was performed in a laminar airflow box (Relatec, Ofterdingen, Germany) under vacuum.

The final preparations consisted of 4 different types of basic ingredients: amino acids, carbohydrates, electrolytes, and lipids. The blending of the compounds was performed under vacuum in a completely closed system. First, half of the volume of the glucose infusion was sucked into the plastic bag through 1 of the plastic tubes, which was connected to the bag. The electrolytes were added to the remaining volume of glucose infusion and then sucked into the plastic bag. Next, amino acids were blended into the obtained solution. Finally, lipids were added to the solution by sucking the lipid emulsions into the plastic bag. Using the right order of blending ensured the homogeneity of the TPN mixtures.

Storage of Prepared TPN Mixtures

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

Particle Size Measurement

Mean size, size distribution, and polydispersity of the emulsion droplets were measured at 25ºC by an advanced technique of photon correlation spectroscopy using a Malvern Zetasizer 4 apparatus (Malvern Instruments, Malvern, UK) with autosizing mode and auto sample time. Analysis of the fluctuations in the intensity of light scattered from particles undergoing random Brownian motion enables the determination of an autocorrelation function $G(\tau)$ that, in effect, is a measure of the probability of a particle moving a given distance in a τ time (τ is the correlation delay time).

$$G_i(\tau) \propto \sum k_i \exp[-\tau / t_{c,i}(a_i)] \quad (1)$$

The relaxation time (t_c) of fluctuations is related to the diffusion coefficient (D) of particles:

$$t_c = 1/DK^2 \quad (2)$$

from which the particle size can be calculated via the Stokes-Einstein equation. K is the wave vector.

By determining the autocorrelation function for the dispersions stored at 2 to 8°C and 37 ± 0.5°C for various times, one can find the diffusion coefficient and the hydrodynamic radii (a_i) of emulsion droplets.

Zeta Potential Measurements

Laser Doppler electrophoresis was used for investigating the surface electric properties of the emulsion droplets. Measurements were performed 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 the Malvern Zetasizer 4 apparatus at 25 ± 1°C, and the resulting frequency spectrum was translated into electrophoretic mobility. Using

Table 1. Composition of the TPN mixtures*

Compounds	Quantity (mL) TPN Mixture 1	TPN Mixture 2
Rindex 10 % (TEVA Ltd, Gödöllő, Hungary) 500.0 mL Magnesium Chloride hexahydrate 0.051 g, Calcium Chloride 0.09 g, Potassium Chloride 0.13 g, Sodium Chloride 1.985 g, Glucose monohydrate 55.0 g	1500	1500
Electrolyte C (University Pharmacy of the Semmelweis University, Budapest, Hungary) 100.0 mL Sodium Chloride 2.13 g, Potassium Chloride 3.39 g, Magnesium sulphate 1.82 g	100	100
Aminoven 10% 500 mL infusion (Fresenius Kabi AB, Uppsala, 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 12.0 g, L-histidine 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	500	500
Intralipid 20% infusion (Fresenius Kabi AG, Bad Homburg, Germany) 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% infusion (Fresenius Kabi AG, Bad Homburg, Germany) Structured triglycerides: 200 g, Purified egg phospholipids: 12 g, Glycerol (anhydrous) (Ph Eur): 22.0 g, Water for injection to 1000 mL	-	500

*TPN indicates total parenteral nutrition.

an AZ 104-type cell, 5 mobility measurements were done on each of 4 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 Smoluchowski 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 analyzer (Radelkis Ltd., Budapest, Hungary).

Dynamic Surface Tension Measurements

The examinations were performed on the day of preparation and after 1, 4, 7, and 10 days. The surface tension of the emulsions was determined by the 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, Lagenbach, Germany) at 25°C ± 0.5°C. The method determines the maximum mass of liquid pulled from the surface by lifting the specified solid (eg, ring or plate). The force (f) measured on the electric balance is the force necessary for lifting out the solid measuring device from the surface of the liquid.

The contact angle can be calculated from the extrapolated buoyancy slope:

$$\cos \Theta = f/p\gamma \qquad (3)$$

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 vapor) of the examined liquid. Three parallel measurements were performed on all 4 kinds of samples.

Statistical Evaluation

Surface tension values measured after different storage intervals were compared with the paired 2-sample *t* test for both kinds of mixtures. The comparison was made between data

Table 2. Ionic Concentrations of the Prepared TPN Mixtures*

Compounds	Concentration (mol/dm^3) in the TPN Mixture
Na^+	0.0545
K^+	0.0212
Mg^{2+}	0.0067
Cl^-	0.0772
SO_4^{2-}	0.0064
Ca^{2+}	0.0009

*TPN indicates total parenteral nutrition.

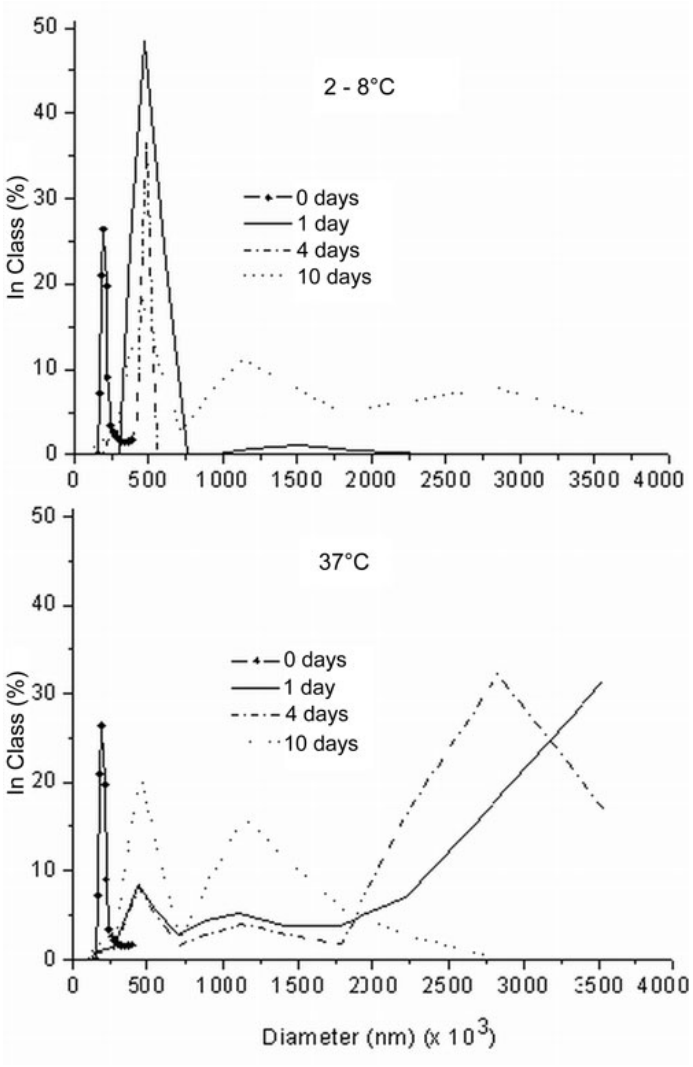

Figure 1. Size distribution functions by volume of total parenteral nutrition 1 emulsions stored for various times at 2 to 8°C and 37 ± 0.5°C.

obtained 1 day after preparation and data obtained after 4, 7, and 10 days. The statistics were calculated using Microsoft Excel 2002.

Results and Discussion

Figures 1 and 2 illustrate the size distribution functions by volume and the mean droplet sizes determined after various times for the 2 TPN emulsions stored at 2 to 8°C and 37 ± 0.5°C, respectively. These results clearly show that the peaks of the distribution functions obtained after longer storage times shift toward larger values, indicating that during storage, the size of the droplets of both emulsions increases while the emulsions become more polydisperse.

By comparing the corresponding size distributions of the 2 emulsions, one can see that the TPN 2 emulsions containing STs exhibited higher kinetic stability. In addition, the rate of droplet coalescence in the emulsions stored at the lower temperatures definitely slowed, especially in the emulsions containing only LCTs. In Figures 3 and 4, the electrokinetic properties of the emulsions are illustrated. The emulsion droplets are negatively charged. The zeta potential of the droplets in the emulsions of original composition is fairly low, mainly because of the high ionic concentration in their media. In a previous study, we found a significant difference between the zeta potential values of LCT- and ST-containing mixtures.[17] In the present emulsions, however, notable differences in the zeta potential of the droplets of the 2 compositions could not be detected even after longer storage times and at both temperatures (peaks of the individual samples overlap). This might be attributed to the higher Na^+ (0.0545 M vs 0.0380 M), Mg^{2+} (0.0067 M vs 0.0039 M), and Ca^{2+} (0.0009 M vs 0.0000 M) content compared with those of the earlier examined mixtures. The higher electrolyte concentrations could have reduced the advantageous effects of the structured lipids on the zeta potential values.

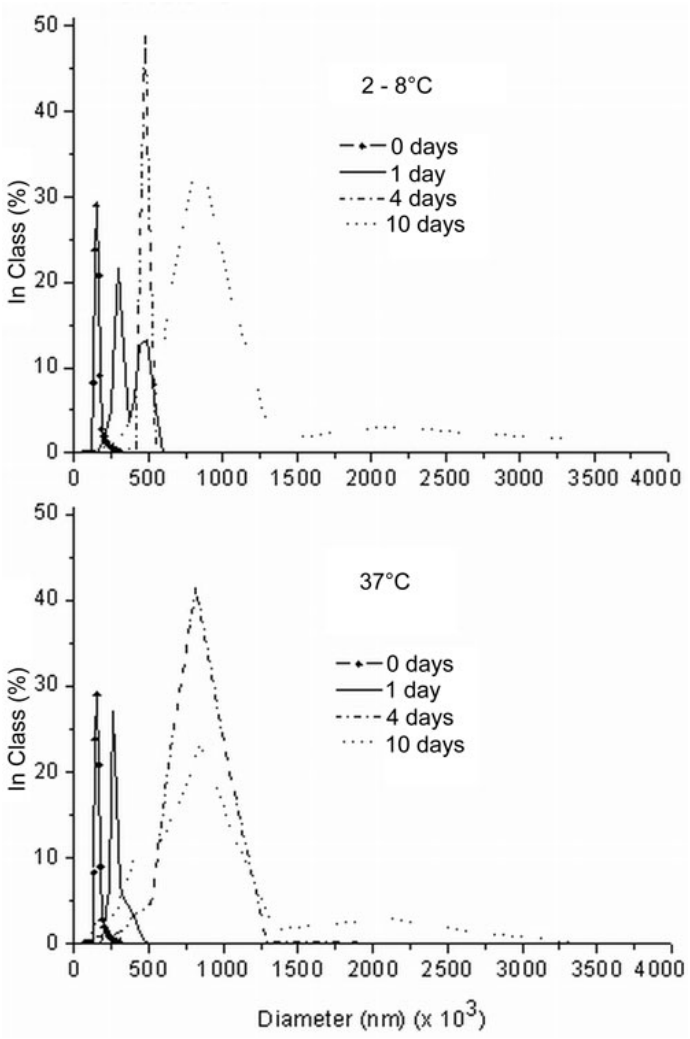

Figure 2. Size distribution functions by volume of total parenteral nutrition 2 emulsions stored for various times at 2 to 8°C and 37 ± 0.5°C.

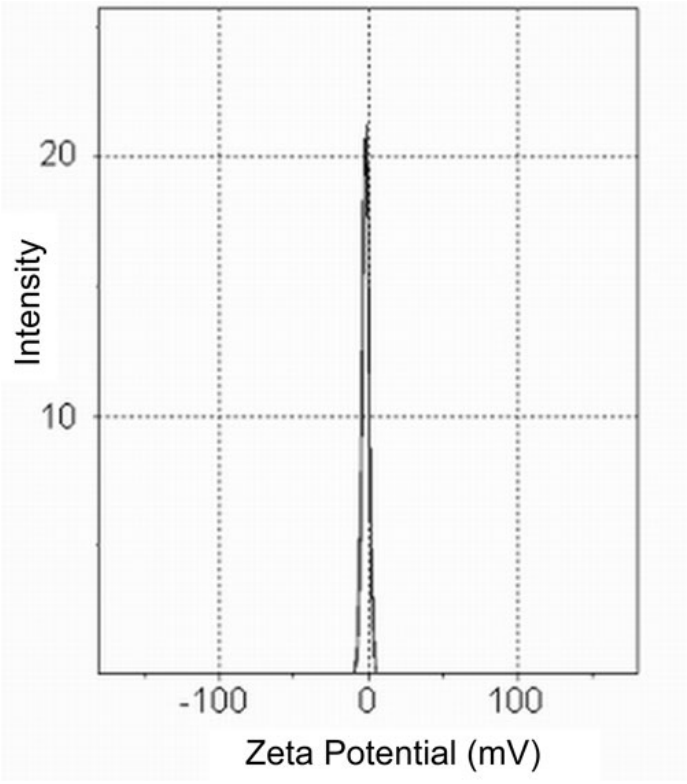

Figure 3. Zeta potential of the droplets in total parenteral nutrition 1 emulsions stored for various times at 2 to 8°C and 37 ± 0.5°C.

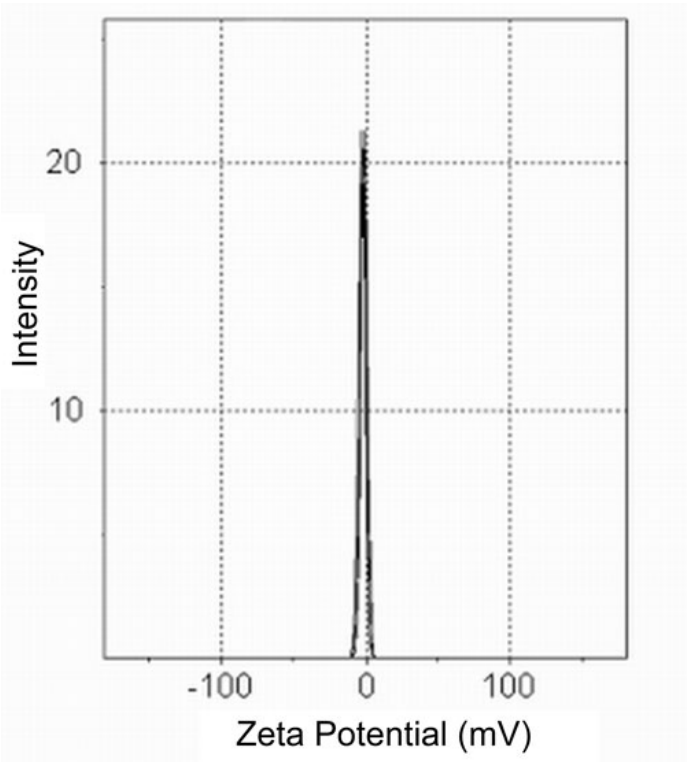

Figure 4. Zeta potential of the droplets in total parenteral nutrition 2 emulsions stored for various times at 2 to 8°C and 37 ± 0.5°C.

Table 3. pH Values of the Mixtures Before and After Storage Under Different Conditions*

Storage Time (days)	pH Structolipid (TPN 1)		pH Intralipid (TPN 2)	
	2-8˚C	37˚C	2-8˚C	37˚C
0	5.90	—	5.95	—
1	5.97	5.92	5.98	5.92
4	5.86	5.83	5.85	5.84
7	5.89	5.86	5.91	5.78
10	6.01	5.94	6.05	5.94

*Average of 3 parallels, SD ≤ 0.25. TPN indicates total parenteral nutrition.

Nevertheless, considerably larger (–17 to –19 mV) zeta potentials were obtained for the droplets in the emulsions diluted 10-fold by distilled water, and the (slightly) higher values were measured in the TPN 2 emulsions.

Since the ionic concentration of the 2 TPN emulsions was equal, and zeta potential and pH values measured in the course of storage (Table 3) did not change markedly, the lower physicochemical stability of emulsions prepared with LCTs cannot 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 the case of STs is responsible for the more efficient stabilization.[17] The latter is responsible for the efficient stabilization, which could be tracked by the different interfacial surface structure of the dispersed droplets.

The surface tension values measured by the Wilhelmy plate operations are summarized in Table 4. The surface tension values determined with the Du Noüy ring correlated well to values measured by the plate method, but the latter resulted in higher accuracy. As can be seen in Table 4, in the case of admixtures containing the structured lipid component, the obtained surface tension values did not show significant changes at 2 to 8°C, indicating a more stable interfacial surface structure. In contrast, the surface tension of emulsions containing exclusively LCTs stored at 2 to 8°C significantly decreased during storage, indicating that there were interfacial structural changes. It is important to note that this method is appropriate for the determination of the tension on the surface of the Wilhelmy plate, not the droplets. Normally, a decrease in surface tension is linked to stabilization, but in this case it meant that structural changes (eg, leaking of surfactants from the droplets) occurred during storage. The samples kept at 37°C presented significant changes (surface tension decreases) in both cases, which suggests that relatively high temperature storage affects the stability of admixtures containing structured lipids as well. In earlier work, we found that the surface tension values of ST-containing mixtures remained constant even at 37°C.[17] The difference between study findings can again be explained by the higher

Table 4. Surface Tension Values of Different Total Parenteral Nutrition Emulsions Stored Under Different Conditions*

	Surface Tension (mN/m)							
	Structolipid				Intralipid			
Storage Time (days)	2-8°C	*P*	37°C	*P*	2-8°C	*P*	37°C	*P*
1	31.44 ± 0.45	—	31.44 ± 0.45	—	30.43 ± 0.42	—	30.43 ± 0.42	—
4	30.18 ± 0.73	>0.01	29.24 ± 0.17	<0.01	30.95 ± 0.65	<0.01	28.98 ± 0.86	>0.01
7	31.18 ± 0.15	>0.01	29.38 ± 0.23	<0.01	27.64 ± 0.16	<0.01	25.99 ± 0.06	<0.01
10	31.19 ± 0.19	>0.01	28.29 ± 0.51	>0.01	27.76 ± 1.06	<0.01	27.13 ± 0.41	<0.01

*Average of 3 parallels, ± SD. *P* refers to the comparison of the surface tension values with the corresponding values obtained after 1 day ($\alpha = 0.01$).

electrolyte content of the emulsions in the present study, because the higher ionic concentrations could have counteracted the stabilizing effect of structured lipids.

The results of the droplet size distribution and surface tension measurements 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.[15] As these authors reported, separate droplets of MCTs and LCTs had poorer physicochemical stability than did droplets 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 favorable interfacial location of STs.

An important finding of the study is that the favorable stabilizing effect of structured lipids can be reduced by the ionic concentration of the media of the emulsions.

CONCLUSIONS

The kinetic stability of total nutrient admixtures containing either LCTs or STs could be compared by the combination of particle size distribution, zeta potential, and dynamic surface tension measurements. Droplet size distribution and surface tension data showed that the emulsions containing structured lipids proved to be more stable, especially at lower storage temperatures. In addition to providing advantageous metabolic effects, STs improve the physical stability of TPN admixtures. Higher electrolyte concentrations of the mixtures can interfere with this stabilizing effect. When alteration of a certain mixture's electrolyte concentration is not possible because of clinical considerations, further stabilization could be achieved using structured lipids.

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