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Investigation of short-term stability of parenteral nutrition nanoemulsions prepared under laboratory conditions

Ispitivanje kratkotrajne stabilnosti nanoemulzija za parenteralnu ishranu izrađenih u laboratorijskim uslovima

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Abstract

Background/Aim. The application of nanoemulsions (NE) in parenteral nutrition represents a very important advancement that marked the medicine and pharmacy of the twentieth century. Over the years, the technology of the production of NE and total parenteral nutrition (TPN) nanoemulsions or admixtures has undergone constant improvement. Representing the continuation of the previous research, this paper deals with nanoemulsions in a concentration of 20% that were prepared under laboratory conditions. The main emphasis was put on the possibility of detecting the potential presence of large droplets or agglomerates of droplets that could cause fatal effects. In addition, the quality assessment of the TPN admixture containing these nanoemulsions was performed. These results were compared with the results obtained from the TPN admixture prepared from the industrial emulsion (Lipofundin MCT/LCT 20%®). **Methods.** During the 30-day period of monitoring nanoemulsion physical-chemical characteristics, the volume diameters that define the width of the lipid droplet size distribution were determined using the laser diffraction method. In addition, TPN physical and chemical characteristics were monitored for 72 hours and included: measurements of the mean droplet diameter, the volume diameter, distribution of the droplet size, ie. polydispersity index (PDI), ζ-potential, and pH values. **Results.** Obtained results were in accordance with the literature data related to the quality of parenteral nanoemulsions (values of volume diameters ranged between 50 and 490 nm). TPN admixtures remained stable during the testing period, even in cases when TPN admixtures containing either a newly formed or an industrial nanoemulsion were tested. **Conclusion.** Characteristics of investigated nanoemulsions do not significantly alter under the ambient temperature storage. If the preparation principles and the component mixing order are followed, TPN admixture possessing satisfactory physical and chemical quality and stability can be obtained.

Key words:
parenteral nutrition, total; nanoparticles; emulsions; quality control.

Apstrakt

Uvod/Cilj. Primena nanoemulzija (NE) za parenteralnu ishranu predstavlja izuzetno značajno dostignuće koje je obeležilo medicinu i farmaciju dvadesetog veka. Tokom godina, tehnologija izrade NE i smeša za totalnu parenteralnu ishranu (TPN) stalno se usavršavala. Ovaj rad predstavlja nastavak prethodnog istraživanja i odnosi se na problematiku nanoemulzija (NE) koncentracije 20%, izrađenih u laboratorijskim uslovima. Osnovni akcenat stavljen je na mogućnost detektovanja eventualnog prisustva većih kapi ili njihovih aglomerata koji bi mogli da izazovu fatalne efekte. Pored toga, izvršena je i procena kvaliteta smeše za TPN sa NE. Rezultati su upoređeni sa rezultatima dobijenim praćenjem smeše za TPN izrađene od fabrički proizvedene emulzije (Lipofundin MCT/LCT 20%®). **Metode.** Primenom metode laserske difrakcije, praćenjem u periodu od 30 dana, dobijeni su rezultati koji se odnose na širinu raspodele veličina kapi NE, izraženu kao volumenski prečnik. Fizičko-hemijske karakteristike smeša za TPN određivane su tokom 72 sata i obuhvatale su: merenje srednjeg prečnika kapi, volumenskog prečnika, distribucije veličina kapi (PDI) i ζ-potencijala, kao i pH-vrednosti. **Rezultati.** Dobijeni rezultati bili su u skladu sa literaturnim podacima o kvalitetu parenteralnih NE (vrednosti volumenskih prečnika kretale su se između 50 i 490 nm). Tokom 72 h praćenja, TPN su ostale stabilne (i smeša za TPN sa NE izrađenom u laboratoriji, kao i TPN sa fabrički izrađenom NE). **Zaključak.** Tokom

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čuvanja u ambijentalnim uslovima, ispitivane karakteristike NE nisu se značajno menjale. Ukoliko se poštuju principi izrade i redosled mešanja komponenti, dobija se smeša za TPN sa zadovoljvajućim fizičko-hemijskim kvalitetom i stabilnosti.

Ključne reči:
ishrana, parenteralna, totalna; koloidi; emulzije; kontrola kvaliteta.

Introduction

The discovery and development of parenteral nanoemulsions represent the milestone and a great achievement in various fields of medicine and pharmacy.

In that sense, one of the most important and most common use of nanoemulsions is for parenteral nutrition of patients with the nonfunctional gastrointestinal tract. Primarily, it has ensured significantly more comfortable and faster way of satisfying patients' requirements for energy, essential fatty acids and fat-soluble vitamins. All of this contributes to the faster recovery and prolongation of life of critically ill patients. For such a purpose, nanoemulsions are used either alone or as a component of the total parenteral nutrition (TPN) admixture.

The spectrum of diseases in which the TPN application is indicated is very wide, so it can be said that they are used in almost all areas of medicine. TPN admixtures known as the "All-in-One", are systems in which all the macronutrients (amino acids, glucose, lipids) and micronutrients (electrolytes, vitamins, oligoelements) are mixed and stored in the ethyl-vinyl-acetate (EVA) bag [1, 2].

From the pharmaceutical aspect, parenteral nutrition nanoemulsions are, by its nature, oil in water emulsions, so called O/W systems. The formation of these colloidal systems is not a spontaneous process, because the additional energy and a surfactant are needed for their production [3].

This research is conducted in line with the general physical and chemical aspects related to nanoemulsions. As far as their stability is concerned, it is widely known that nanoemulsions are thermodynamically unstable and kinetically stable.

In the first rough calculation, the mathematical model that proves the thermodynamic instability of nanoemulsions uses the expression for the change of the modified Gibbs function ("free energy") which is expressed through enthalpy (H), absolute temperature (T), entropy (S) and the work (W) done to increase the surface of oil droplets [4]:

$$\Delta G = \Delta H - T\Delta S + W$$

Analyzing the changes of particular terms in this equation during the nanoemulsion production process, it comes out that ΔG is a positive value. From the physical-chemical aspect, it is known that the system is moving toward the equilibrium position (stability) only in the case when the Gibbs function decreases [5–7]. Thus, the fact that stems from this is that nanoemulsions are unstable in terms of thermodynamics.

The assertion that nanoemulsions are kinetically stable refers to the information on the speed at which the destabilization processes occurs. Changes of critical nanoemulsion parameters take place very slowly, so the system remains in the achieved metastable state for a significantly long period of time.

Not only from the stability aspect, but also from the medical, ie. safety aspect, it is of particular importance to investigate whether the droplet size distribution of nanoemulsions contains droplets that can be fatal for a patient.

Considering capillary dimensions, the presence of particles larger than 5 μm would not be desirable as they can cause fat embolism [8, 9]. In addition, according to the United States Pharmacopeia (USP) Chapter 729 requirements, the mean droplet size of the parenteral nanoemulsion must be < 500 nm [10].

In the literature, the combination of several measurement techniques is recommended for measuring the mean size of lipid droplets (the hydrodynamic droplet diameter) [11, 12].

In order to detect the possible presence of larger droplets or agglomerates of droplets, the aim of this study was to assess, from the droplet size distribution aspect, the quality of produced nanoemulsions. During the experiment preparation stage and the result processing stage, the method of the 2^{4-1} fractional factorial design was used, and the results were compared with the results for droplet sizes of one of industrial nanoemulsions [13].

The next important aim of the research was to evaluate how nanoemulsion prepared under laboratory conditions affects characteristics of the TPN admixture when that nanoemulsion is mixed with above mentioned TPN admixture ingredients. Our further task was also to prepare the TPN admixture that contains an industrial nanoemulsion, and then to compare the obtained results.

Methods

Materials

The oil phase was composed of the following components: soybean oil, Lipoid Purified Soybean Oil 700 (SO) and egg phospholipids with 80% phosphatidylcholine, Lipoid® E80 – EP (both from Lipoid GmbH, Germany), fish oil, oleum jecoris (Ph. Eur. 7.5) – FO, Miglyol 812®, medium-chain triglycerides (MCT), and antioxidant (α-tocopherol), all from Caelo, GmbH, Hilden, Germany, and the second antioxidant, thioglycolic acid (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The water phase was composed of Lipoid Sodium Oleate B (Lipoid GmbH, Germany), Kolliphor® P 188 (Poloxamer 188) – Pl, (BASF, Ludwigshafen, Germany), glycerol (Ph. Eur.) and sodium hydroxide (Ph. Eur.) (Merck, Germany). Water used in the experiment was double distilled and obtained from the Milli Q-water purification system (Millipore, MA).

Selection of ingredients was carried out in accordance with their acceptability for parenteral administration.

In addition, for the production of TPN admixtures, the following solutions were used: the amino acid solution, Aminoven 10%® (Fresenius Kabi, Austria), Glucosi injection 50%® (S.A.L.F., Italy), a fat emulsion – Lipofundin® MCT/LCT 20% (Braun, Germany), electrolytes – Potassium chloride 7.45%® (Braun, Germany), Sodium chloride 10%® (Fresenius Kabi, Deutschland), Calcium-Sandoz 10%® (Novartis, Switzerland), Magnesium chloride injection 200 mg/mL® (Milan, Ireland), Glycophos® (Fresenius Kabi, Austria), as well as water for injection a 500 mL (Braun, Germany).

Methods

The oil and water phases were prepared separately. The oil phase (20% w/w) was heated to the temperature of around 65–70 °C, under mild stirring with a magnetic stir bar (IKA RCT Basic, Germany) at 800 rpm for 15 minutes until the surfactants were completely dissolved. In another plate, water soluble components were measured and heated to the same temperature as the oil phase during permanent stirring. Then, the water phase was added slowly to the oil phase with constant stirring, and that mixture was pre-emulsified by the Ultra-Turax T_{25} high shear mixer (Janke & Kunkel Ika- Labortechnik, Germany) at 13500 rpm for five minutes. In that way, a crude emulsion with the droplet size of around 2 μm was obtained.

In the second production phase (the high-pressure homogenization), the nanoemulsion droplets were obtained by processing a crude emulsion through the high-pressure homogenizer (Gea Niro Panda plus 2000, Italy). During that experimental phase, the pressure was 300 and 700 bars, while the number of homogenization cycles was four and ten. These values varied according to the mentioned 2^{4-1} fractional factorial design (Table 1).

The temperature of the entire homogenization process was maintained at 40 °C. The procedure was repeated as many times as defined by the experimental design (Table 1). Independent parameters included the type of oil phase (*X1*), the surfactant (egg phospholipids) with or without the second surfactant – Poloxamer 188 (*X2*), the number of homogenization cycles (*X3*) and the process pressure (*X4*), while the dependent parameter was the volume droplet size (*y*). In accordance with this type of design, it was possible to calculate only the selected interaction terms.

Table 1

Experimental matrix according to the 2^{4-1} fractional factorial design

Formulation	X_1	X_2	X_3	X_4
1	A1	1	4	300
2	A2	1	4	700
3	A1	2	4	700
4	A2	2	4	300
5	A1	1	10	700
6	A2	1	10	300
7	A1	2	10	300
8	A2	2	10	700

X_1 – oil phase (A1 – mixture of FO and SO, A2 – mixture of FO and MCT);
X_2 – surfactant (1 – EP, 2 – mixture of EP and Pl);
X_3 – number of cycles; X_4 – pressure (bar).
For abbreviations see under Table 2.

Obtained values were fitted into the following model:

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4$$

The signs b_0 to b_4 represent regression coefficients that demonstrate the influence of independent variables on the dependent variable; thereby b_0 is the intercept and b_1, b_2, b_3 and b_4 are the linear coefficients of the respective independent variables. On the other hand, b_{12}, b_{13} and b_{14} are regression coefficients that demonstrate the interaction between corresponding variables associated with respective model factor interactions (X_1X_2, X_1X_3, X_1X_4).

According to the design used in this study, the composition of prepared nanoemulsions is shown in Table 2.

The prepared nanoemulsions were subjected to the following tests: visual examination, centrifugation, then the repeated visual examination following the centrifugation, and finally the volume droplet size measurement by laser diffraction method.

In the course of testing, the samples were stored at the room temperature (about 25 °C). The dynamics of the nanoemulsion quality monitoring was the following: the first measurement was performed immediately after their preparation (0h), the next measurements were done after 10 and 30 days.

Table 2

Composition of nanoemulsion formulations

Formulation	Composition of nanoemulsion formulations (%, w/w)									
	SO	FO	MCT	EP	Pl	SOl	G	Toc	TA	Water to [g]
1	10	10	—	1.20	—	0.03	2.50	0.01	0.01	100
2	—	10	10	1.20	—	0.03	2.50	0.01	0.01	100
3	10	10	—	1.20	0.60	0.03	2.50	0.01	0.01	100
4	—	10	10	1.20	0.60	0.03	2.50	0.01	0.01	100
5	10	10	—	1.20	—	0.03	2.50	0.01	0.01	100
6	—	10	10	1.20	—	0.03	2.50	0.01	0.01	100
7	10	10	—	1.20	0.60	0.03	2.50	0.01	0.01	100
8	—	10	10	1.20	0.60	0.03	2.50	0.01	0.01	100

SO – soybean oil; FO – fish oil; MCT – medium-chain triglycerides; EP –egg yolk phospholipids; Pl – Poloxamer 188; SOl – sodium oleate; G – glycerol; Toc – α-tocopherol; TA – thioglycolic acid.

Centrifugation of prepared nanoemulsions

The reason for centrifugation comes from the fact that the phase separation process can best be determined by exposing nanoemulsions to the high speed centrifugation, so all of the prepared nanoemulsions were subjected to the centrifugation at 3750 rpm/min for five hours, with the centrifuge radius of 10 cm ("Heraeus Megafuge 16 Centrifuge" – Thermo Fisher, Germany). The fact that this manner of centrifugation corresponds to the effect of one-year gravity refers to classical emulsions [14], but there are not any data on its possible application on the nanoemulsions.

Volume weighted diameters

The laser diffraction (LD) method was applied in this study for the detection of possible droplet agglomerates. For that purpose, the Cilas Granulometer device (1090 LD, France) was used with the measurement range of 20 nm–500 μm. In order to avoid the effects of multiple light scattering, each sample of prepared nanoemulsions was diluted with highly purified water to an appropriate concentration that the aparatus detected as optimal for the measurement. The values thus obtained refer to the volume distribution of particles and represent mean values of three repeated measurements. Using this method, the volume diameters were obtained by measuring the angle of light scattering based on the Mie theory. It should be mentioned here that the volume diameter is defined as the percentage of the presence of particle for a given volume that has a smaller size than a given value. The measurement of the industrial nanoemulsion droplet size (Lipofundin® MCT/LCT 20%) was performed in the same way.

The concept of the *Span* is introduced as an additional parameter for the width of the size distribution. The *Span* of the volume-based size distribution is defined as:

$$Span = [d(0.9) - d(0.1)] / d(0.5)$$

and gives an indication of how far the 10 percent *d(0.1)* and 90 percent *d(0.9)* points are apart, normalized with the midpoint *d(0.5)*[15]. The small *Span* value indicates the narrow size distribution.

Preparation and characterization of TPN admixtures

The preparation of TPN admixture from produced nanoemulsion was carried out as follows: after 30 days in storage, one nanoemulsion (NE I) selected by a random sampling was incorporated into the composition of the TPN admixture together with other ingredients. This nanoemulsion had the following characteristics: the mean droplet diameter 183.1 ± 1.6, the polydispersity index (PDI) 0.067 ± 0.006 and ζ-potential – 33.2 ± 1.4. The same procedure was followed for the industrial nanoemulsion (NE II – Lipofundin® MCT/LCT 20%). Its characteristics were: the mean droplet diameter 268.8 ± 4.5, PDI 0.073 ± 0.036 and ζ-potential – 29.7 ± 0.7. The composition of the both admixtures (the amount of macroingradients – amino acids, glucose, and fat, as well as the amount of electrolytes – Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-}) is shown in Table 3. It was formulated on the basis of daily requirements of patients requiring intravenous nutrition[16].

Table 3
Composition of total parenteral nutrition (TPN) admixtures

Ingredients	Units	Volume
Aminoven 10%®	mL	25
Glucosi injectio 50%	mL	15
Lipid nanoemulsion (NE I or NE II)	mL	12.5
Water for injections to	mL	100
Potassium	mmol	3
Sodium	mmol	3
Magnesium	mmol	0.2
Calcium	mmol	0.2
Chlorides	mmol	5.9
Phosphates	mmol	0.25
Total volume of the mixture	mL	100
Energy value of the mixture	kJ	273
Nitrogen	g	0.4
Amino Acids	g	2.5
Glucose	g	7.5
Lipids	g	2.5

NE I – laboratory nanoemulsion; NE II – industrial nanoemulsion.

The admixtures were disposed into the sterile EVA infusion bag according to standard operating procedures [17, 18]. Samples of the prepared admixtures were taken from each bag at the defined time intervals (0h, and then after 24 and 72 hours storage at the temperature of 2–8 °C), the dynamics of which was determined based on the essential health needs of hospitalized patients. In practice, TPN admixtures are prepared on a daily basis except for weekends and holidays, when there is a need to use them 72 hours after the preparation (during that time the admixtures are kept at 2–8 °C). The monitoring of the TPN admixture quality included the visual examination, the measurement of the mean droplet diameter, PDI, the ζ-potential, the volume diameter of particle size and pH value.

In this case, the measurement of the mean droplet diameter, the PDI index and the ζ-potential was carried out using light scattering method. Furthermore, the diameter of particle size was additionally measured by the LD method. The determination of pH-value for admixtures was carried out by the potentiometric method using the calibrated pH-meter (Mettler Toledo, Seven Go, Swiss).

Results

Characterization of nanoemulsions

From the visual criteria view point, during and at the end of testing, all prepared nanoemulsions as well as TPN admixtures (which are also considered as nanoemulsions

from the pharmaceutical aspect), retained a milky-white appearance with a bluish shade, what is typical for these systems. In addition, neither the phase separation nor the formation of cream, coalescence, or the phase inversion was observed in the samples tested after centrifugation. The thin layer of oil was separated only in nanoemulsions numbered 1 and 3 (Table 2), but it was redispersed by stirring.

Volume weighted diameters

When the LD technique was used for particle sizing, d(0.1) diameter was found to be about 50 nm for all investigated samples (Table 4). Moreover, d(0.5) the range of values was from 130 to 290 nm, and finaly d(0.9) ranged from 220 to 490 nm. Particles above 500 nm were not observed what signified that obtained nanoemulsions were suitable for parenteral nutrition [10]. The Table 4 shows the values of the droplet volume diameter and Span of the prepared nanoemulsions. For example, if d(0.1) as in this table is 50 nm, it means that, in a given volume of the sample, 10% of the particles have a diameter of less than 50 nm, the d(0.5) of 160 nm indicates that 50% of the particles have a diameter of less than 160 nm, while d(0.9) of 400 nm indicates that 90% of particles has the diameter of less than 400 nm, etc. [15].

Table 4

Volume diameters (d) in nm and Span values of laboratory made nanoemulsions

Formulation*	Immediately after preparation				after 10 days				after 30 days			
	d(0.1)	d(0.5)	d(0.9)	Span	d(0.1)	d(0.5)	d(0.9)	Span	d(0.1)	d(0.5)	d(0.9)	Span
1	50	160	400	2.19	60	170	420	2.12	60	220	490	1.95
2	60	150	320	1.73	50	150	380	2.20	60	210	450	1.86
3	60	130	250	1.46	60	130	220	1.23	80	200	380	1.50
4	60	130	230	1.31	60	130	230	1.31	50	150	380	2.20
5	60	150	320	1.73	50	160	400	2.19	60	170	420	2.12
6	60	140	290	1.64	60	160	410	2.19	60	210	450	1.86
7	60	130	230	1.31	60	130	230	1.31	60	140	290	1.64
8	50	130	220	1.31	60	130	230	1.31	60	130	230	1.31

***see Table 2.**

In Tables 5, 6 and 7, this issue was clarified by presenting the effect of independent variables and, especially their interactions on dependent variables, namely d(0.1), d(0.5), and d(0.9).

Table 5 clearly shows that none of the independent variables had a significant effect on the dependent variable, which, in this case, represented the droplet size distribution expressed in the form of d(0.1).

Based on the results presented in Table 6, it is evident that the surfactant type and amount had the greatest effect on d(0.5). Namely, when both surfactants are used, the value of d(0.5) was reduced. This effect was more pronounced after storage (after 30 days, it was –23.75). Other factors, as well as the interaction among the factors, did not significantly affect this level of droplet size distribution.

Table 5

Regression coefficients demonstrating the influence of independent variables and the interaction between corresponding variables on a volume diameter – d(0.1)

Variable	Immediately after preparation	10 days after preparation	30 days after preparation
X_1	+1.25	0	-3.75
X_2	+1.25	+2.5	+1.25
X_3	+1.25	0	-1.25
X_4	+1.25	-2.5	+3.75
X_1X_2	-1.25	0	-3.75
X_1X_3	-1.25	+2.5	+3.75
X_1X_4	-1.25	0	-1.25

***X₁* – oil phase; *X₂* – surfactant; *X₃* – number of cycles; *X₄* – pressure.**

Table 6

Regression coefficients demonstrating the influence of independent variables and the interaction between corresponding variables on a volume diameter – d(0.5)

Variable	Immediately after preparation	10 days after preparation	30 days after preparation
X_1	-2.5	-2.5	3.75
X_2	-10	-15.0	-23.75
X_3	-2.5	0	-16.25
X_4	0	-2.5	-1.25
X_1X_2	+2.5	+2.5	-11.25
X_1X_3	0	+2.5	+11.25
X_1X_4	+2.5	0	-3.75

***X₁* – oil phase; *X₂* – surfactant; *X₃* – number of cycles; *X₄* – pressure.**

Table 7

Regression coefficients demonstrating the influence of independent variables and the interaction between corresponding variables on a volume diameter – d(0.9)

Variable	Immediately after preparation	10 days after preparation	30 days after preparation
X_1	-17.5	-2.5	-8.75
X_2	-50	-87.5	-66.25
X_3	-17.5	2.5	-38.75
X_4	-5	-7.5	-16.25
X_1X_2	10	5	-6.25
X_1X_3	7.5	5	1.25
X_1X_4	10	0	-21.25

***X₁* – oil phase; *X₂* – surfactant; *X₃* – number of cycles; *X₄* – pressure.**

According to obtained results, the surfactant type and amount had the greatest effect on d(0.9) as it was shown that with the increase of the value of that independent variable, the value of d(0.9) decreased. This effect was particularly pronounced 10 days after the preparation (the regression coefficient amounted to –87.5). The number of homogenization cycles significantly affected d(0.9), too. The negative sign in front of the regression coefficient indicates the antagonistic effect of this factor, ie. the fact that the increase in the number of homogenization cycles results in the decrease in the d(0.9) value.

The frequency distribution of nanoemulsion droplet sizes is presented by a cumulative frequency curve (Figure 1).

The abscissa shows a specific droplet size interval (in microns, μm), while the ordinate shows the percentage of certain fractions, that is, the percentage of droplets smaller than the monitored ones. The cumulative curve represents the determined droplet size as well as all the droplets smaller than the determined ones. In addition, the logarithmic scale is used on the abscissa to clearly present a wide range of results, while the linear scale is used on the ordinate.

Characterization of the total parenteral nutrition admixtures

The measurement results of the mean droplet diameter, the PDI, the ζ-potential and pH value of the TPN admixture containing a nanoemulsion prepared under laboratory conditions and the admixture containing an industrial nanoemulsion are given in Table 8, while the results of the droplet volume diameter measurement are shown in Table 9.

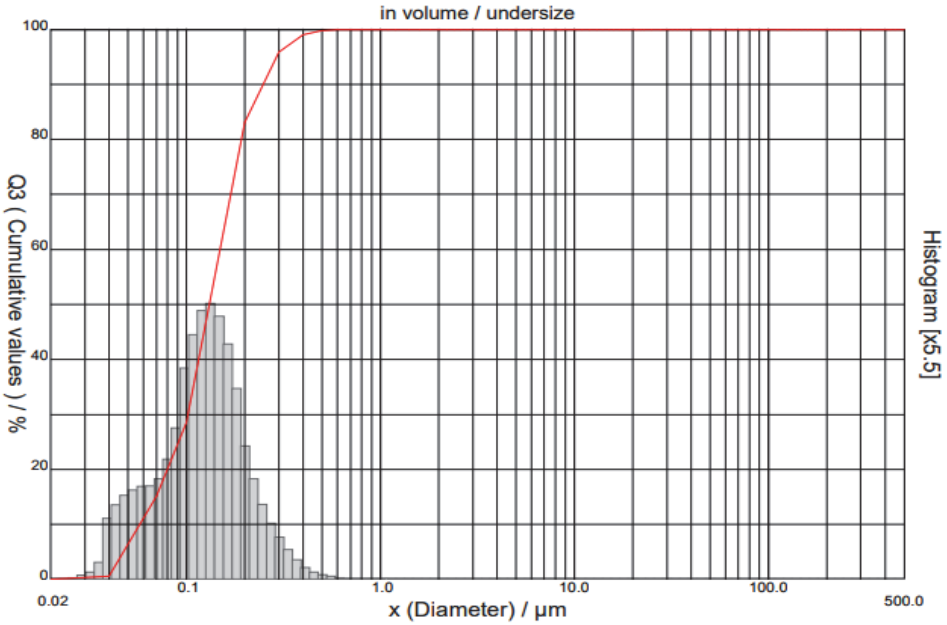

Fig. 1 – Cumulative frequency curve.

Table 8

Characteristics of the total parenteral nutrition (TPN) admixtures

TPN admixture	Mean droplet diameter (nm)			PDI			ζ-potential			pH-value		
	0h	24h	72h	0h	24h	72h	0h	24h	72h	0h	24h	72h
PN $_{I}$	185.67	214.51	271.03	0.111	0.099	0.202	-34.7	-43.2	-43.5	6.05	5.90	5.85
TPN $_{II}$	208.47	272.70	291.77	0.120	0.144	0.226	-47.3	-44.5	-38.5	5.60	5.45	5.60

TPN $_{I}$ – admixture that contains a laboratory-made nanoemulsion (NE I); TPN $_{II}$ – admixture containing an industrial nanoemulsion (NE II); PDI – polydispersity index.

Table 9

Volume diameters (d) in nm and Span values of the total parenteral nutrition (TPN) admixtures

TPN admixture	Immediately after preparation				After 24h				After 72h			
	d(0.1)	d(0.5)	d(0.9)	Span	d(0.1)	d(0.5)	d(0.9)	Span	d(0.1)	d(0.5)	d(0.9)	Span
TPN $_{I}$	60	160	320	1.62	60	210	380	1.52	60	210	410	1.67
TPN $_{II}$	50	150	380	2.20	60	280	410	1.25	60	240	480	1.75

TPN $_{I}$ – admixture that contains a laboratory-made nanoemulsion (NE I); TPN $_{II}$ – admixture containing an industrial nanoemulsion (NE II).

Discussion

Characterization of nanoemulsions for parenteral nutrition

Droplet size and droplet size distribution are the most important parameters that characterize the quality of nanoemulsions for parenteral use. With the LD method applied in this study, it is possible to see that the creation of agglomerate drops does not take place, indicating, thus, that there is no occurrence of neither coalescence nor Ostwald ripening, which represent the most common forms of nanoemulsion destabilization [19, 20]. Of particular importance here is to identify the presence of droplets larger than defined by the USP. The obtained results are in accordance with the USP 729 requirements (the mean droplet size less than 500 nm) [10, 21].

The distribution of droplet sizes represented the droplet volume diameter in all of the tested samples was relatively uniform, and the measured values were higher compared to the values obtained by the light scattering method [13]. The reason for that is that LD method gives the volume-based droplet size distribution, while the light scattering measurement is based on the intensity of light.

Analyzing the data given in the Table 4, it can clearly be seen that the values for droplet volume diameters measured in the nanoemulsion samples number 1, 2, 5 and 6 were the highest, particularly in cases d(0.9). So, their values immediately after the preparation were 400, 320, 320 and 290 nm, respectively, while at the end of the testing period they were 490, 450, 420 and 450 nm, respectively. Since these nanoemulsions contained a lower concentration of surfactants ie., that the only egg phospholipids were used for their production, it can be concluded that these facors had the greatest effect on the droplet volume diameters. Data presented in Table 4 also shows that the increase in the surfactant concentration significantly reduced the droplet size, what is in accordance with theoretical settings [22–24], as well as with the results obtained in our previous research [13]. According to the named after Derjagmin, Landau, Verwey, Overbeek (DLVO) theory, a surfactant forms a film around the emulsion droplet, and, with increasing the potential energy for repulsing droplets, provides its protection. On the contrary, the absence of a surfactant causes strengthening of the van der Waals attraction forces due to the increase in the potential energy associated with the gravitational potential energy. However, the increased values of volume diameters given in Table 4 were within stated limits required by the literature [10, 21].

The values obtained from *Span* measurement show that, during the testing, nanoemulsions numbered 7 and 8 had the narrowest width of the volume diameter distribution.

When analyzing the regression coefficients, it can be seen that the type and concentration of surfactant, ie. the factor X_2, had relatively the greatest impact on the volume diameter of nanoemulsion droplets. That is especially evident in the case of $d(0.9)$ analysis (Table 7). The X_2 value is negative, what means that droplets were smaller in size when the absolute value of the regression coefficient was higher, what was especially observed after monitoring nanoemulsions for 10 and 30 days.

In the case of d(0.1) analysis, Table 5 shows that none of the factors had a significant impact, while the analysis of d(0.5) given in Table 6 shows that the factor X_2 had the greatest impact. In addition, to make changes related to the volume diameter more obvious, the graphical presentation of one of the representative results is given for illustration purposes.

It is a common practice to use egg phospholipids as an emulsifier for the industrial parenteral nutrition nanoemulsions. However, there are no reports in the literature on the use of the combination of egg phospholipids and Poloxamer 188. It was interesting to examine what impact the combination of electrostatic emulsifier (egg phospholipids) and the stern emulsifier (Poloxamer 188) has on the emulsification and characterization of prepared nanoemulsions, what was actually done in this research.

It was shown that when those two emulsifiers were combined (samples no. 3, 4, 7 and 8), the volume diameters, and therefore, the Span values were lower (Table 4). According to the authors' knowledge, there are no data in the available literature on the production and testing of short-term stability of nanoemulsions for parenteral nutrition, which would be prepared under laboratory conditions.

Characterization of the total parenteral nutrition admixtures

Problems associated with the stability of TPN admixtures are complex and occur due to their complex composition. In addition, it is known that electrolytes, particularly polyvalent cations, can cause the occurrence of various forms of physical and chemical instability. The fact that these complex systems may also contain more than 50 components indicates a greater possibility of the occurrence of a number of instabilities and different unwanted incompatibilities.

Determination of conditions under which the stability of the TPN admixture is maintained is considered a serious problem because seemingly unnoticeable changes can take place in them slowly and over a long period of time. In addition, if a phase separation occurs, the problem that arises is the formation of seemingly homogeneous emulsion by redispersion of separated droplets of the size that may be fatal for a patient.

The results obtained by measuring the basic physical values that are characteristic for the TPN admixtures will be further discussed. Measurement results in our study confirm the stability of prepared admixtures for the total parenteral nutrition (TPN_I and TPN_{II}). As indicated, the mean droplet diameter, the polydispersity index, the ζ-potential, the volume diameter of droplets, and pH value were measured.

As for the mean droplet size, the results show that there are no significant differences between values obtained by measurements immediately after the TPN admixture preparation and those obtained after 24 and 72 hours. C omparison of the mean droplet sizes of the TPN admixture produced with the nanoemulsion prepared under laboratory conditions (TPN_I), and the TPN admixture containing the industrial nanoemulsion (TPN_{II}) shows that the values of mean droplet diameters of the admixture containing the industrial nanoemulsion (NE II) were higher.This can be explained by the

fact that the industrial nanoemulsion NE II (Lipofundin® MCT/LCT 20%) was produced much earlier than the one produced under the laboratory conditions. (NE I). This shows the effect of so-called „natural aging" on the quality of nanoemulsion, and, by that, on the quality of the TPN admixture. Generally speaking, when the "natural aging" of nanoemulsions is concerned, their stability is explained by the ability of very small droplets to reduce the effect of the gravitational force through the Brownian motion. However, the nanoemulsion stability can be compromised in another way, that is, by the influence of some external factors (so-called induced aging). The most common factors are: the type and concentration of electrolytes that are added to the admixture, the admixture pH value, the temperature and other.

On the day when the TPN admixture formulations (TPN_I and TPN_{II}) were prepared, they contained droplets with the average size of 185.67 nm and 208.47 nm, and with a very narrow PDI amounting up to 0.111 and 0.120. The mean droplet diameters after 24 hours of the admixture storage at the room temperature (214.51 and 272.70 nm) were slightly different from those measured at the time zero. Furthermore, the admixtures were stored in the refrigerator, and after 72 hours, the mean diameter of the lipid droplets was measured again (271.03 and 291.77 nm, respectively). It is observed that even these values did not exceed the established USP limits, ie. they all were less than 500 nm [10, 21].

As it is known from the literature, the values obtained by measuring the PDI provide information of the deviation from the average droplet size. In all the measurement intervals, these values were in accordance with theoretical settings which, from the perspective of the droplet size distribution, define the quality of parenteral nanoemulsions. Namely, it is considered that if the PDI was less than 0.25, such a preparation is suitable for the parenteral administration [25]. This indicates that all the admixtures were homogeneous.

Apart from the fact that the ζ-potential should not always be a key indicator of the colloidal system stability [26], it is known that if these values are higher than 30 mV, such a preparation can be used for the parenteral nutrition. In general, the values of ζ-potential are conditioned by the amount of added electrolytes (especially cations), and pH values. The reduction of pH value points to the release of free fatty acids what leads to the increased ζ-potential negative values (it is considered a favorable factor that affect the increase in stability) [27, 28]. However, in this case, it cannot be taken into account.

There are no large differences between pH values of prepared TPN admixtures. During the testing period, the values obtained by measuring pH value of the TPN_I admixture ranged from 6.05 to 5.85, ie. pH value was found to be slightly acidic after 72 hours. As for the TPN_{II} admixture, the values after the admixture preparation and after 72 hours were 5.6. This decrease in pH value was insignificant, and triglycerides did not decompose into free fatty acids.

It is well known that at low pH values (about 2.5), the phase separation of the admixture takes place. Glucose solutions have acidic pH (3.5 to 6.5), and, therefore, should not be directly mixed with the fat emulsion because low pH values cause the reduction of the fat drop surface potential, what would further lead to the emulsion phase separation. Because of that, glucose solutions are, firstly, mixed with amino acid solutions, which by its buffering capacity resist changes in pH values, and then a nanoemulsion is added to such a mixture. Here, it should be said that amino acids exert their protective influence on the stability by mechanical impact, ie. through insertion into the intermediate layer between the oil and water phase of the emulsion, and, thus, prevents the integration of drops [12, 16]. By slow homogenization of the prepared admixture, an equilibrium dispersion state is created (homogeneous admixture).

Values obtained by measuring the volume diameter and *Span* of the tested TPN admixtures also confirmed their stability during 72 hours. During that period, neither droplet agglomerates were formed nor droplets with a diameter greater than 500 nm were detected.

Conclusion

The research results showed that no droplet aggregates were observed in the short-term period of monitoring of the prepared nanoemulsions (immediately after, and 10 and 30 days after preparation), that is, the processes of destabilization did not occur. Namely, the values of volume diameters – d(0.1), d(0.5), and d(0.9) were within the established USP limits (≤ 500 nm). Results for the nanoemulsions prepared in the laboratory setting had approximately the same values as for the industrial nanoemulsion.

Regarding the preparation and characterization of TPN admixtures, no significant differences were found among parameters (mean droplet diameter, PDI, ζ-potential, pH value, volume diameter) measured during the 72-hour monitoring period.

Finally, the study showed that nanoemulsions can be successfully produced under laboratory conditions. These nanoemulsions with their composition and physical-chemical characteristics obtained from the short-term monitoring period are suitable for parenteral feeding. They can also be used as a component of the TPN admixture that is safe for the administration in the hospital setting.

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REFERENCES

1. *Mirković D, Ibrić S, Antunović M.* Quality assessment of total parenteral nutrition admixtures by the use of fractional factorial design. Vojnosanit Pregl 2013; 70 (4): 374–9.
2. *Austin P, Stroud M.* Prescribing Adult Intravenous Nutrition. 1st ed. London: Pharmaceutical Press; 2007.
3. *Wabel C.* Influence of lecithin on structure and Stability of Parenteral Fat Emulsions [dissertation]. Nürnberg: Universität Erlangen; 1998.
4. *Lawrence J.* Disperse systems. In: *Denton P, Rostron C,* editors. Pharmaceutics: The Science of Medicine Design. Oxford: Oxford University Press; 2013. p. 180–1.
5. *Tadros T, Izquierdo P, Esquena J, Solans C.* Formation and stability of nanoemulsions. Adv Colloid Interface Sci 2004; 108–109: 303–18.
6. *Kozić Đ.* Thermodynamics – principles and applications. 2nd ed. Belgrade: Faculty of Mechanical Engineering, University of Belgrade; 2012. (Serbian)
7. *Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM.* Nanoemulsions: formation, structure, and physical properties. J Phys Condens Matter 2006; 18: R635–66.
8. *Jumaa M, Müller BW.* The effect of oil components and homogenization conditions on the physicochemical properties and stability of parenteral fat emulsions. Int J Pharm1998; 163(1–2): 81–9.
9. *Benita S, Levy MY.* Submicron emulsions as colloidal drug carriers for intravenous administration: Comprehensive physicochemical characterization. J Pharm Sci 1993; 82(11): 1069–79.
10. *United States Pharmacopeia 39 and National Formulary 34* (USP39–NF34). Globule size distribution in lipid injectable emulsions. Rockville, MD: The United States Pharmacopoeial Convention; 2016.
11. *Ball PA.* Methods of assessing stability of parenteral nutrition regimens. Curr Opin Clin Nutr Metab Care 2001; 4(5): 345–9.
12. *Washington C, Athersuch A, Kynoch D.* The electrokinetic properties of phospholipid stabilized fat emulsions. The effect of glucose and pH. Int J Pharm 1990; 64: 217–22.
13. *Mirković D, Ibrić S, Balanč B, Knez Ž, Bugarski B.* Evaluation of the impact of critical quality attributes and critical process parameters on quality and stability of parenteral nutrition nanoemulsions. J Drug Deliv Sci Technol 2017; 39: 341–7.
14. *Rungseevijitprapa W, Siepmann F, Siepmann J, Paeratakul O.* Disperse Systems. In: *Florence IA, Siepmann J*, editors. Modern Pharmaceutics. New York: Taylor & Francis Group; 2010. p. 398.
15. *Hamishehkar H, Emami J, Najafabadi AR, Gilani K, Minaiyan M, Mahdavi H* et al. The effect of formulation variables on the characteristics of insulin-loaded poly(lactic-co-glycolic acid) microspheres prepared by a single phase oil in oil solvent evaporation method. Colloids Surf B Biointerfaces 2009; 74(1): 340–9.
16. *Sobotka L, Allison S, Fürst P, Meier R, Pertkiewicz M, Soeters P.* Basics in clinical nutrition. 3rd ed. Prague: House Galén; 2004.
17. *McKinnon BT.* FDA safety alert: hazards of precipitation associated with parenteral nutrition. Nutr Clin Pract 1996; 11(2): 59–65.
18. *Mirtallo J, Canada T, Johnson D, Kumpf V, Petersen C, Sacks G,* et al. Task force for the Revision of Safe Practices for Parenteral Nutrition. Safe practices for parenteral nutrition. JPEN J Parenter Enteral Nutr 2004; 28(6): S39-70.
19. *Taylor P.* Ostwald ripening in emulsions. Colloids Surf A Physiochem Eng Asp 1995; 99(Suppl 2–3): 175–85.
20. *McClements DJ.* Edible nanoemulsions: fabrication, properties, and functional performance. Soft Matter 2011; 7(6): 2297–316.
21. *Driscoll DF.* Commercial lipid emulsions and all-in-one mixtures for intravenous infusion – composition and physicochemical properties. World Rev Nutr Diet 2015; 112: 48–56.
22. *Tadros TF.* Emulsion stability. In: *Becher P*, editor. Encyclopedia of Emulsion Technology. New York: Marcel Dekker; 1983. p. 129–285.
23. *Trotta M, Pattarino F, Ignoni T.* Stability of drug-carrier emulsions containing phosphatidylcholine mixtures. Eur J Pharm Biopharm 2002; 53(2): 203–8.
24. *Han F, Li S, Yin R, Liu H, Xu L.* Effect of surfactants on the formation and characterization of a new type of colloidal drug delivery system: Nanostructured lipid carriers, Colloids Surf A Physiochem Eng Asp 2008; 315(1–3): 210–6.
25. *Müller RH, Schmidt S, Buttle I, Akkar A, Schmitt J, Brömer S.* SolEmuls® – novel technology for the formulation of i.v. emulsions with poorly soluble drugs. Int J Pharm 2004; 269: 293–302.
26. *Klang V, Matsko N, Raupach K, El-Hagin N, Valenta C.* Development of sucrose stearate-based nanoemulsions and optimization through γ-cyclodextrin. Eur J Pharm Biopharm 2011; 79(1): 58–67.
27. *Benita S, Levy MY.* Submicron emulsions as colloidal drug carriers for intravenous administration: comprehensive physicochemical characterization. J Pharm Sci 1993; 82(11): 1069–79.
28. *Rozentur E, Nassar T, Benita S.* Materials for nanoemulsions and their influence on the biofate. In: *Torchilin V, Amiji MM,* editors. Handbook of materials for nanomedicine. Singapore: Pan Stanford Publishing Pte. Ltd.; 2010. p. 515–54.

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