ORIGINAL ARTICLE

UDC: 616/083.2/003:611.1]:[615.451:615.014.4
DOI: 10.2298/VSP1304374M

Quality assessment of total parenteral nutrition admixtures by the use of fractional factorial design

Analiza kvaliteta smeša za totalnu parenteralnu ishranu primenom delimičnog faktorijalnog dizajna

Dušica Mirković*, Svetlana Ibrić†, Mirjana Antunović*‡

***Pharmacy Department, Military Medical Academy, Belgrade, Serbia;**
†Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia;
‡Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia

Abstract

Background/Aim. Parenteral nutrition as a specific aspect of providing nutritients still remains a permanent topic of both theoretical and experimental research. Total parenteral nutrition (TPN) admixtures have complex contents making difficult to maintain their stability. The most critical parameter is the diameter of a lipid droplet, i.e. droplet size distribution. It is recommended that droplet size should not be more than 5 μm and that the presence of greater droplets should not exceede the value of 0.05%. Lipid droplets size is affected particularly by electrolyte addition, especially polyvalent cations. There is a danger of the added electrolytes interaction with lipid droplets which leads to their aggregation and negative effects upon the admixtures stability. The aim of this study was to assess the effect of added electrolyte and lipid phase quantity on the admixture stability. **Methods.** Electrolytes were added to the studied admixture of a defined basic formulation contents in accordance with recommendations from the literature. Droplets size measurements were performed using the method of laser diffraction with a laser particles analyzer. Effects of independent variables were calculated and evaluated using commercial software. Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations, as well as the quantity of fat phase were chosen as studied factors, i.e. independed variables. The system response, or dependent variable was the median of droplets size. Each of the factors was varied at two levels, higher (+1) and lower (-1), according to the 2^{5-2} fractional factorial design. **Results.** The study suggested the presence of relative uniformity of the results of all the measurements regardless of the quantity of added electrolytes and lipid phase. It was shown that undoubtedly there is the influence of 2-valent cations (calcium and magnesium) upon lipid droplets size, which is in a direct correlation with theoretical assumption. **Conclusion.** Within a 72-hour testing period there was no significant increase in droplet size, i.e. the studied admixtures remained stable considering droplet size median as the criterion of stability.

Key words:
parenteral nutrition; particle size; fat emulsions, intravenous; electrolytes; quality control.

Apstrakt

Uvod/Cilj. Parenteralna ishrana, kao specifičan vid nadoknade hranljivih materija, i dalje predstavlja stalnu temu teorijskog i eksperimentalnog izučavanja. Složeni sastav smeše za totalnu parenteralnu ishranu (TPI) otežava održanje njihove stabilnosti. Najkritičniji parameter je dijametar lipidnih kapi, odnosno raspodela veličina kapi. Postoji preporuka da veličina kapi ne bi trebalo da prelazi 5 μm i da zastupljenost većih kapi ne prelazi vrednost od 0,05%. Na veličinu lipidnih kapi poseban uticaj ima dodavanje elektrolita, naročito viševalentnih katjona. Postoji opasnost da dodati elektroliti interreaguju sa lipidnim kapima, što dovodi do njihovog spajanja i ima negativan uticaj na stabilnost smeša. Cilj ovog rada bio je da se istraži kako dodati elektroliti i količina lipidne faze utiču na stabilnost ovih smeša. **Metode.** Ispitivanoj smeši sa definisanim sastavom osnovne formulacije izrađenoj u bolničkoj apoteci, dodavani su elektroliti na osnovu preporuka iz literature. Merenje veličine kapi vršeno je metodom laserske difrakcije pomoću laserskog analizatora čestica. Uticaj nezavisno promenljivih je procenjen i izračunat primenom komercijalnog softvera. Kao nezavisno promenljive, u svojstvu ispitivanih faktora izabrani su koncentracije Na^+, K^+, Ca^{2+} i Mg^{2+}, kao i količina masne faze. Odgovor sistema, ili zavisno promenljiva veličina, bila je medijana veličine kapi. Svaki od faktora variran je na dva nivoa, gornji (+1) i donji (–1), odnosno primenjen je 2^{5-2} frakcioni fak-

Correspondence to: Dušica Mirković, Pharmacy Department, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 11 3608 382. E-mail: ducim@eunet.rs

torijalni dizajn. **Rezultati.** Istraživanje je pokazalo da nezavisno od količine dodatih elektrolita i količine lipidne faze postoji relativna ujednačenost rezultata za sva merenja. Analiza pojedinačnih faktora ukazuje na nesumnjiv uticaj dvovalentnih katjona (kalcijuma i magnezijuma) na veličinu lipidnih kapi, što je u direktnoj korelaciji sa teoretskim postavkama. **Zaključak:** Tokom ispitivanog 72-časovnog perioda nije bilo značajnog povećanja veličine kapi, odnosno ispitivana smeša ostala je stabilna sa stanovišta medijane veličine kapi kao kriterijuma stabilnosti.

Ključne reči:
ishrana parenteralna; čestice, veličina; emulzije, masne; intravenske; elektroliti; kvalitet, kontrola.

Introduction

A major requirement to meet the safe and efficient use of total parenteral nutrition (TPN) admixtures concerns their stability, i.e. unchangeability over time. They are oil-in-water type emulsions (O/W) and containing more than 50 components (amino acids, carbohydrates, lipids, electrolytes, vitamins, oligoelements, insulin, heparin and water for injections). It is obviously difficult to obtain and maintain their quality, especially due to the fact that these components could interact, while these interactions are not visible.

The most critical parameter that could adversely affect the stability of TPN admixtures and endanger their suitability for the clinical use is the lipid droplet diameter. Numerous factors affect lipid droplet size, thus consequently, the stability of these emulsion systems. It is significant that lipid droplet size is particularly affected by electrolyte addition, especially by multivalent cations. However, while an admixture is required to contain electrolytes for the organism to maintain normal functioning, if high quantities of ions are added (which is the case in patients affected by metabolic disorder) it induces danger of their interaction with lipid droplets resulting in increasing of droplets size and their aggregation and imposing negative effect upon the stability of admixture [1].

It has to be emphasized here that the droplets of fat are usually not of the regular spherical shape differing from each other almost always in size, so that polydispersity is present in each sample. It is, therefore, more reasonable to refer to "droplet size distribution" than to "droplet size", these two parameters, however, being most often regarding as identical [2, 3]. It comes out that the most significant parameters to follow, except for the homogeneity, are the size stability, droplet size distribution, and even (uniform) distribution of individual droplets within the emulsion.

Attempts to introduce droplet (globule) size measurement technique into the emulsions quality analysis began in the 80s of the 20 century. It was then suggested the lipid droplet diameter to be 0.5–1 μm that is approximately equal to the diameter of endogenous lipids (chyllomicrons), while the cut-off droplet size to be 5 μm that was not a real requirement considering that there was no sophisticated measuring technique. It is not until 2004 that the United States Pharmacopeia (USP) introduced two criteria: globule size should not be more than 5 μm, and the presence of globules larger than that size should not exceed 0.05% [2, 4].

Emulsions are known to belong to the group of colloidal systems. Yet, the droplet size in emulsions could not be determined by means of the methods usually used to determine the droplet size in colloids. The reason for that being the fact that the droplets in emulsions are deformable and tend to aggregate into large droplets. This affects light scattering and light refraction and other phenomena using to determine the size of droplets [5].

It is well-known that there is no unique reliable method for measuring the size of emulsions droplets in a wide spectrum. Methods of measuring droplets, starting from classical to the most recent – modern ones, have been selectively used to cover a certain diapason of requirements and range of measuring [6, 7]. Any methods are specific in their own way, have their disadvantages and limitations and cover a certain range of the droplets size that could be determined by them. Besides, neither of the methods could determine a full droplets size distribution (starting from a few nm to many μm). The method of laser diffraction [8–10] is the most often used one. It is also the one used in this study.

The use of mathematical, statistical and other models make it possible to predict, that is to choose experimental settings. Although numerous, and titled as experimental design, the most often used are the methods of so-called factorial design. Factorial design is applied to determine the influence of certain factors on the system, giving the possibility to assess which of the factors exerts the most significant influence. Factorial design application provides a considerably high number of information on the studied system by a relatively low number of experiments [11, 12].

The aim of the study was to determine the impact of concentration and type of electrolytes, as well as a lipid phase on the size of lipid droplets by the use of experimental design as regard to the change of values ranging from the lowest to the highest ones, as required by the practice itself. The experimental design here helps understand the way the said factors influence the median value as the numerically and statistically significant characteristics of experimentally obtained values.

Methods

The studied TPN admixtures were prepared in the hospital pharmacy using the techniques of aseptic procedures in a laminar chamber. The study was performed in various time intervals: immediately after the preparation – 0 h, and after 12 h, both at the temperature of 25°C. Time of 12 h responds to the time of application TPN admixture to a patient. Next, the compounds were kept at the temperature spanning from 2°C to 8°C and analyzed after 72 hours.

Table 1 shows the basic formulation composition of a TPN compound, while Table 2 shows the independent variables, i.e. component values of various quantities (concen-

Table 1

The composition of a total parenteral nutrition (TPN) admixture

Components	TPN admixture quantity (mL)
Amino acids as Vamin 18* (nitrogen 18 g/L, amino acids 114g/L)	500
Glucose infundible 200 mg/mL**	1000
Intralipid 20%*	250 and 500

*Fresenius Kabi; **Pharmacy Department, Military Medical Academy

Table 2

Real values of the independent variables

Independent variable	Lower level (−1)	Upper level (+1)
Na^+ concentration (mmoL), X_1 (Natrii chloridi inj. 100 mg/mL*)	50	150
K^+ concentration (mmoL), X_2 (Kalii chloridi inj. 74.5 mg/mL*)	75	100
Ca^{2+} concentration (mmoL), X_3 (Calcii chloridi inj. 100 mg/mL*)	10	20
Mg^{2+} concentration (mmoL), X_4 (Magnesii sulfatis inj. 250 mg/mL*)	10	20
Lipid phase quantity (g), X_5, (Intralipid 20%**)	50	100

*Pharmacy Department, Military Medical Academy; **Fresenius Kabi

tration of the added electrolytes and the quantity of the lipid component).

The quantity of electrolytes added to the admixtures for TPN was determined on the basis of daily requirements by patients with the increased need for electrolytes (for example polytraumatized patients) in compliance with the guidelines from the literature [13].

The particle size was determined by a laser particle analyzer (Microtrac Fra 9200, Leeds & Northrup) which uses laser diffraction technique. Measurements were repeated three times for each sample. Visual analysis was used to observe flocculation and creaming of the emulsion after admixture [14].

As mentioned above, the most significant parameters to control, besides homogenity, are the stability of the size and droplet size distribution, as well as the uniformity of individual droplet size distribution within an emulsion. Considering numeric characteristics of random variables (a diameter of lipid droplets) it is common to define a certain characteristic size which indicates a kind of medium or orientational value around which any experimentally obtained value for the random variable are grouped. The median random variable was used in this study.

The median is a value defined as specifically divide a numerical series in two equal parts. One part includes any elements of the value equal or less than the median, while the other includes these of the equal or greater than the median. In that sense, in a sequence of values set by the size (”arranged sequence“), the median values are found exactly in the middle, thus they are also called ”50th percentile“ [15, 16].

In setting the experimental conditions, as well as in analyzing the results obtained by the experiment, to calculate factorial effects the analysis of variance (ANOVA) method was used, performed using the computer program Design Expert [12, 17]. The chosen factors, namely independent variables, were as follows: Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations, as well as the quantity of lipid phase. The system response, or dependent variable size was the median. The design, referred as 2^3, was chosen because according to the theory to obtain the response in the assessments like this one at least 8 experiments have to be performed. If each of the 5 factors would vary at 2 levels we could obtain a total factorial design type 2^5, thus in 32 experiments we would often obtain insignificant effects of higher order of magnitude.

Each of the factors ($X_1 – X_5$) was varied at two levels, namely higher (+1) and lower (-1), that is the fractional factorial design $2^{5–2}$ was applied. The responses were put into the mathematical model of the first-order polynomial including 5 variables and a constant member b_0 (further on referred to as constant) [12].

$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5$

y - response (in this case the median of the droplet size),

b_0 - coefficient (Table 4). The constant b_0 is an average response for given in the any experiments, i.e. average effect of any factors; for the given experiment $b_0 = 1 / 5_i = {}_I\Sigma^5 y_i$,

b_1, b_2, b_3, b_4, b_5 - factorial effects,

X_1, X_2, X_3, X_4, X_5 – independent variables.

Results

There was no visual change in color, clarity, creaming or precipitates in the studied TPN during the study period.

To simplify factorial effects analysis, the parameters to verify are marked as coded (Table 3) presents experimental matrix of 2^{5-2} fractional factorial design; values of independent variables are presented in coded values. Table 3 also shows the values of median obtained by data processing of droplet sizes measured by a Microtrac Fra 9200.

The results indicate a relative uniform data for all the measurements regardless the quantity of electrolytes and the quantity of lipid phase in the studied compounds for TPN. There were no significant differences in droplets size distribution. Also, in all the measurements done the median values were not more than 0.6 μm. This fact implies that the com-

Table 3

Experimental matrix of 2^{5-2} experimental design; [values of independent variables are presented in coded values and system responses droplet sizes median (μm) after composing the preparation (0 h) and after 12 h and 72 h]

Number of samples	X_1	X_2	X_3	X_4	X_5	System responses y_1	y_2	y_3
1	−1	−1	−1	+1	+1	0.354	0.451	0.484
2	+1	−1	−1	+1	−1	0.241	0.429	0.524
3	−1	+1	−1	−1	+1	0.231	0.251	0.507
4	+1	+1	−1	−1	−1	0.292	0.250	0.479
5	−1	−1	+1	−1	−1	0.296	0.444	0.469
6	+1	−1	+1	−1	+1	0.494	0.485	0.445
7	−1	+1	+1	+1	−1	0.342	0.474	0.393
8	+1	+1	+1	+1	+1	0.231	0.515	0.417

Symbols in Table 3:
X_1 – concentration of Na^+; X_2 – concentration of K^+; X_3 – concentration of Ca^{2+}; X_4 – concentration of Mg^{2+}; X_5 – lipid phase quantity; −1 means minimum, +1 means maximum; y_1, y_2, y_3 – lipid droplets size median (μm) in 0 h, 12 h and 72 h

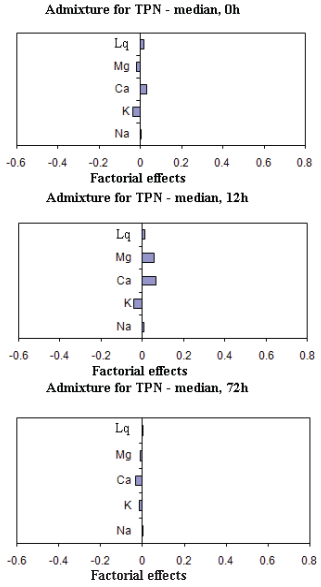
pounds within the whole period of testing, i.e. in a 72-hour period, were stable as regard to the droplet size median as the stability criterion.

Factorial effects analysis

Factorial analysis was done in order to assess which of the chosen factors significantly affect the median.

As displayed in the Table 4, it is obvious that the values of the regression coefficient during the time period in which the mixtures were analyzed had been the greatest when it is the question of the impact of calcium. These results are in direct correlation with theoretical postulations of the influence of polyvalent cations upon the droplets size increase [18]. However, immediately after the production of TPN mixture (0 h), the value of the regression coefficient shows that the impact of potassium is very similar to the value showing the influence of calcium.

Factorial effects can be presented graphically as Pareto chart (Figure 1).



Lq – lipid phase quantity

Fig. 1 – Pareto chart (median of droplets size)

Table 4

The calculated factorial effects and the applicable constants when droplet size median was followed up as a response

Parameters	0 h	12 h	72 h
Coefficient (b_0)	0.31000	0.4100	0.470
Independent variable		Factorial effects	
X_1	0.00437	0.0074	0.00143
X_2	-0.03600	-0.0400	-0.01600
X_3	0.03100	0.0670	-0.03400
X_4	-0.01800	0.0550	-0.01000
X_5	0.01700	0.0130	-0.00143

X_1 – concentration of Na^+; X_2 – concentration of K^+; X_3 – concentration of Ca^{2+}; X_4 – concentration of Mg^{2+}; X_5 – lipid phase quantity

Discussion

Since the beginning of using TPN admixtures, i.e. within the last 30 years, numerous studies on their stability [9, 19–21] have been performed. The majority of authors have suggested that the stability of each admixture has to be verified and that the obtained results cannot be generally accepted as well. Special attention has to be paid to limitations regarding the electrolyte addition, especially polyvalent cations.

Regardless the mentioned facts no clear guidelines have been defined for the maximal quantity of electrolytes that could be added. Due to the need to daily prepare TPN admixtures customized to the requirements of each patient it is necessary to study this problem in details.

Electrolytes addition to a TPN admixture, as mentioned above, disturbs its stability, that is to say it causes physicochemical changes. There are two kinds of interactions between electrolytes and fat globule surface: non-

specific and specific adsorption. Non-specific adsorption occurs when added monovalent cations Na^+ and K^+ are adsorbed at the surface of fat droplets. At high electrolyte concentrations, and above the critical flocculation concentration (CFC), electrostatic repulsive forces decrease their value thus, becoming equal to the Van der Waals attractive forces, and at a certain moment, the flocculaton process of an emulsion starts. Another adsorption kind, that, besides electrostatic interactions, involves the chemical interaction of ions and the surface of droplets is a so-called specific adsorption. It is the chemical process of adsorption occurring between polyvalent cations (Ca^{2+} and Mg^{2+}) and lipid droplets which forms a bridge with the anionic emulgator component of the two lipid droplets. It, thus, leads to the increase of the droplet size and the occurrence of various types of instability such as aggregation, coalescence, flocculation, phase separation, and emulsion phase inversion as well. In the terminal stage of destabilisation of the emulsion, a droplets size could range from 5 μm to 50 μm which is not allowed in parenteral emulsions. It has been shown that a higher concentration and valency of cations cause the destabilisation of the emulsion to a higher extent [21].

In planning the experiment, we started from the fact that the number of factors that could affect the stability of an emulsion for parenteral nutrition is high, thus it was necessary to choose adequate factors for the study. This choice was based on the previous experiences with this issue assessed both theoretically and practically. We also considered all the known results obtained in the earlier studies [12, 22]. In designing the research, as well as in the results processing, we considered the correlation between theoretical assumptions and the obtained results applicability in pharmacy and the clinical practice.

Since the levels of independent variables are marked by coded values, absolute values of regression coefficients directly give information about their influence upon the studied system. The higher absolute value of the regression coefficient, the higher the influence of the corresponding independent variable. If the regression coefficient has the sign of ”+“, or ”-”, the increase in the level of independent variable conditions increase, or decrease in the dependent variable, that is the studied system response.

Conclusion

The results of this study suggest that the assessed TPN admixture could be used within a 72-hour period (duration of the analysis). No significant increase in a droplet size was observed. Considering the droplet size median as the criterion of stability, the studied admixtures remained stable. Thus, this study also clarified how the changes in values of individual factors influence the system to respond. It could be concluded that the metod of the fractional factorial design is suitable for planning a trial and assessing the obtained results.

REFERENCES

1. *Sobotka L, Allison S, Fürst P, Meier R, Pertkiewicz M, Soeters P.* Basics in clinical nutrition. 4th ed. Prague: House Galén; 2011.
2. *Driscoll DF.* Globule-size distribution in injectable 20% lipid emulsions: Compliance with USP requirements. Am J Health Syst Pharm 2007; 64(19): 2032−6.
3. *Mehta RC, Head LF, Hazrati AM, Parr M, Rapp RP, DeLuca PP.* Fat emulsion particle-size distribution in total nutrient admixtures. Am J Hosp Pharm 1992; 49(11): 2749−55.
4. *Driscoll DF.* Lipid injectable emulsions: Pharmacopeial and safety issues. Pharm Res 2006; 23(9): 1959−69.
5. *Đaković Lj.* Colloid chemistry. 4th ed. Belgrade: Zavod za udžbenike i nastavna sredstva; 2006 (Serbian)
6. *Ball PA.* Methods of assessing stability of parenteral nutrition regimens. Curr Opin Clin Nutr Metab Care 2001; 4(5): 345−9.
7. *Driscoll DF.* The significance of particle-sizing measurements in the safe use of intravenous fat emulsions. J Disp Sci Technol. 2002; 23: 679−87.
8. *Sforzini A, Bersani G, Stancari A, Grossi G, Bonoli A, Ceschel G C.* Analysis of all-in-one parenteral nutrition admixtures by liquid chromatography and laser diffraction: study of stability. J Pharm Biomed Anal 2001; 24(5−6): 1099−109.
9. *Lee MD, Yoon JE, Kim SI, Kim IC.* Stability of total nutrient admixtures in reference to ambient temperatures. Nutrition 2003; 19(10): 886−90.
10. *Driscoll DF, Etzler F, Barber TA, Nehne J, Niemann W, Bistrian BR.* Physicochemical assessments of parenteral lipid emulsions: light obscuration versus laser diffraction. Int J Pharm 2001; 219(1−2): 21−37.
11. *Li LC, Sampogna TP.* A factorial design study on the physical stability of 3-in-1 admixtures. J Pharm Pharmacol 1993; 45(11): 985−7.
12. *Ibrić S.* Development of mathematical theory of experiments in pharmaceutical technology. Belgrade: Konstisi; 2006. (Serbian)
13. *Kasper LD, Braunwald E, Fauci SA, Hauser LS, Longo LD, Jameson LJ,* editors. Harrison’s Principles of Internal Medicine. 16th ed. New York: McGraw-Hill; 2005.
14. *Driscoll DF.* Total nutrient admixtures: theory and practice. Nutr Clin Pract 1995; 10(3): 114.
15. *Torbeck LD.* Pharmaceutical and Medical Device Validation by Experimental Design. New York, NY: Informa Healthcare; 2007.
16. *Witting H.* Mathematische Statistik I. Parametrische Verfahren bei festem Stichprobenumfang. Stuttgart: B. G. Teubner; 1985.
17. *Zhang X, Li S.* The orthogonal saturated effects model and its statistical analysis. Chinese J Appl Probabil Statistcs 2009; 25(3): 309−19.
18. *Driscoll DF, Nehne J, Peterss H, Klutsch K, Bistrian BR, Niemann W.* Physicochemical stability of intravenous lipid emulsions as all in one admixtures intended for the very young. Clin Nutr 2003; 22(5): 489−95.
19. *Driscoll DF.* Stability and compatibility assessment techniques for total parenteral nutrition admixtures: setting the bar according to pharmacopeial standards. Curr Opin Clin Nutr Metab Care 2005; 8(3): 297−303.

20. *Washington C.* Stability of lipid emulsions for drug delivery. Adv Drug Deliv Rev 1996; 20(2): 131−45.
21. *Mirković D, Antunović M, Putić V, Aleksić D.* Assessment of the stability of admixture for total parenteral nutrition prepared in the hospital pharmacy. Vojnosanit Pregl 2008; 65(4): 286−90. (Serbian)
22. *Mirković D.* Impact of electrolyte concentration on the characteristics of admixture for total parenteral nutrition [thesis]. Beograd: Military Medical Academy; 2008 (Serbian)

Received on October 10, 2011.
Revised on February 23, 2012.
Accepted on February 29, 2012.