Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 5 pp. 843–849, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

EFFECT OF STORAGE TEMPERATURE ON THE STABILITY OF TOTAL PARENTERAL NUTRITION ADMIXTURES PREPARED FOR INFANTS

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Abstract: Physical, chemical and microbiological stability of total parenteral nutrient (TPN) admixtures was studied as a function of storage time and temperature. Particle size analysis and zeta potential measurements were carried out to evaluate the possible changes in the kinetic stability of the emulsions as a function of storage time and temperature. The concentration changes of the applied additives, those of the ascorbic acid and L-alanyl-L-glutamine, were also determined under different storage conditions. Our results indicate that there were no significant differences in the particle size and zeta potential values of admixtures stored at the three examined temperatures. The best results were obtained in the case of admixtures stored at 30°C temperature. Rapid decomposition of vitamin C was found while the glutamine showed adequate stability as a function of storage time and temperature. According to the results of the physicochemical examinations 10-day storage period of this type of TPN admixtures can be accepted at room temperature. Their storage does not require refrigeration (2-8°C) thus they can be administered without special preheating ensuring better physiological tolerance. Ascorbic acid can be added to the system preceding the administration to the patient because of its rapid decomposition.

Keywords: kinetic stability, all-in-one parenteral nutrition admixture, droplet size distribution, ascorbic acid, L-alanyl-L-glutamine

In the clinical practice, a number of diseases are known that require parenteral nutrition of the patients. Parenteral nutrition should be able to cover the energy needs of the organization and to it should provide the stability of fluids and electrolytes. Accordingly, for parenteral nutrition admixtures contain amino acids, glucose, electrolytes, and fat emulsion in addition to the required vitamins and trace elements. The nutrients can be administered separately, but most often the all-in-one mixtures are applied, taking into account the clinical and economical aspects. However, in contrast to these benefits, a strong limiting factor is the lowering of stability of these multicomponent systems (1). Total Parenteral Nutrition (TPN) admixtures are heterogeneous disperse systems, lipid emulsions, which are thermodynamically unstable (2). Their composition is adjusted to the therapeutic requirements and consequently individualized therapy could be assured to the patients (3). The large number of components and the changing ratio of the additives greatly affect the stability of TPN emulsions (4). Vitamin preparations can protect intravenous lipid emulsions from peroxidation. The administration of multivitamins with the intravenous lipid emulsions provides a practical way to reduce peroxidation of the lipid while limiting vitamin loss (5, 6).

The most critical parameter is the physical stability of systems (2, 7, 8), including the droplet size of the emulsions. If the droplet size exceeds the size of erythrocytes (6-8 μm), embolism can occur, which is of fatal consequences. The droplet size is influenced by several factors; e.g., the added electrolytes by changing the charge of the droplet surface (zeta potential). The droplets due to their surface charge repel each other, thus impeding the coalescence of the droplets (9). In the course of the stability tests phase separation can be observed, but it can be eliminated by simple mechanical influences, although this state is followed by coalescence as

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well (4). The droplet size and droplet size distribution can be usually determined by dynamic light scattering principle operating methods and information can be derived from the charging of the surface by measuring the electrophoretic mobility (zeta potential), that also provides information about the kinetic stability of the system (9, 10).

Chemical degradation is the most important cause of vitamin losses in TPN admixtures. The latter was tracked by the concentration changes of ascorbic acid and L-alanyl-L-glutamine. Vitamins have been mostly quantified in several matrices by HPLC or capillary electrophoresis (11, 12). Few HPLC methods have been developed for simultaneous determination of water- and fat-soluble vitamins (13, 14). The ascorbic acid of parenteral solution was determined by HPLC with UV detection (15). Literature shows that the concentration of ascorbic acid in parenteral solution is out of the acceptable limit (> 90%) (to 82% and 87%) after 48 h at room temperature (25°C). Ascorbic acid was determined after ultrafiltration of the parenteral solution samples by tandem mass spectrometer (ESI-MS/MS) with a minor modification of the literature method (16). The stability assay took 14 days at three temperatures (2-8°C, 25°C, 30°C).

The immune response in critically ill patients is often impaired. Since the glutamine stimulates the transport of nitrogen and reduces the catabolism of the proteins in intestinal or skeletal muscle, therefore glutamine is quickly becoming essential ones due to its increased degradation, thus patients in critical conditions must get exogenous glutamine supplementation.

From the point of practical and safety reasons it could simplify the handling of these systems in the hospital wards without the use of special storage conditions.

Therefore, the primary aim of this study was to investigate the effect of storage temperature on the kinetic stability of the system characterizing by the average particle size, particle size distribution and zeta potential. Another aim of the paper was to examine the degradation of those components sensitive to degradation (ascorbic acid and the glutamine) with monitoring their concentration changes at different temperatures (2-8°C, 25°C, 30°C) for 14 days.

EXPERIMENTAL

Materials

Table 1 summarizes the composition of the prepared TPN admixtures. Ascorbic acid, L-alanyl-L-glutamine, glycylglycine and 3-hydroxy-butyric acid standard were purchased from Sigma-Aldrich (purity > 99.0%).

Table 1. Composition of the TPN admixtures.

Components of the TPN admixture	Packaging [mL]	Quantity (mL)
Infusio glucosi 20% (University Pharmacy, Semmelweis University, Budapest, Hungary)	500	470
Peditrace N inj. (Fresenius Kabi AB, Uppsala, Sweden)	10 × 10	10
Calcimusc inj. (Ca-gluconate 10%, Gedeon Richter Plc., Budapest, Hungary)	10 × 5	20
NaCl 10% inj. (TEVA Pharmaceutical Works Ltd., Debrecen, Hungary)	10 × 10	32
KCl 10% inj. (Pharmamagist Ltd., Budapest, Hungary)	100 × 10	27
Soluvit N inj. (Fresenius Kabi AB, Uppsala, Sweden)	10 × 10	10
Aminoven infant 10% (Fresenius Kabi AB, Uppsala, Sweden)	100	100
Dipeptiven inf. (alanyl-glutamine dipeptide 20%) (Fresenius Kabi Deutschland GmbH)	50	25
Panangin inj. (452 mg potassium aspartate (anhydrous) and 400 mg magnesium aspartate (anhydrous) per 10 mL vial) (Gedeon Richter Plc., Budapest, Hungary)	5 × 10	15
Glucose-1-Phosphate inj. (Fresenius Kabi Austria GmbH, Graz, Austria)	5 × 10	7
Smoflipid 20% inj. (Fresenius Kabi AB, Uppsala, Sweden)	100	100
Vitalipid infant inj. (Fresenius Kabi AB, Uppsala, Sweden)	10 × 10	10

Zeta-potential measurements

Zeta potential measurements were carried out at 25°C using Zetasizer Nano ZS apparatus (Malvern Instruments, UK). An electric field was applied to the dispersion of particles, which would then move with a velocity related to their zeta potential. This velocity was measured using a patented laser interferometric technique called M3-PALS (Phase Analysis Light Scattering). This enables the calculation of electrophoretic mobility, and from this the zeta potential for the accurate measurement using the Smoluchowsky formula and expressed in mV. Measurements were carried out at 25 ± 1°C with the freshly prepared emulsions and also, with the samples stored for 0 to 14 days on 3 different temperatures. Particle size range for zeta potential measurement is from 5 nm to 10 μm.

The evaluation of zeta potential measurements indicates the instability of these systems with the values smaller than ±30 mV. The results showed decreasing tendency especially in samples stored at low temperature.

Particle size measurements

Mean droplet size (MDS), size distribution and polydispersity of the emulsion droplets were measured at 25°C using Zetasizer Nano ZS apparatus (Malvern Instruments, UK).

Dynamic Light Scattering (DLS) is used to measure particle diameter. This technique measures the diffusion of particles moving under Brownian motion, and converts this to size and a size distribution using the Stokes-Einstein relationship. Non-Invasive Back Scatter technology (NIBS) is incorporated in the instrument to give the highest sensitivity simultaneously with the highest dynamic size and concentration range. It includes 2 angle size measurements for the enhanced detection of aggregates, measurement of small or dilute samples, or at high concentration. In addition, the optics is not in contact with the sample and hence the detection optics are said to be non-invasive. The measurement position within the cuvette of the Nano ZS is automatically set to accommodate the requirements of high sensitivity or high concentration. This position is changed by moving the focusing lens.

Particle size range for size measurement is from 0.6 nm to 6 μm.

Statistical evaluation

Average particle size and zeta-potential values 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 infusions stored at different temperatures.

The statistics were calculated using Microsoft Excel 2003.

Determination of the ascorbic acid concentration

The measurement was run by the internal standard method with 3-hydroxy-butyric acid internal standard. Ascorbic acid was diluted in deionized water to obtain solutions at appropriate concentrations for implementation. They were freshly prepared before use. The samples were ultrafiltrated with 10 kDa filter (Millipore) at 14000 rpm before measuring them. The ESI-MS/MS measurements were accomplished with API 4000 QTRAP MS/MS mass spectrometer equipped with Perkin Elmer 200 LC. The ESI-MS/MS system was used in negative ion mode, the quantification of the ascorbic acid ($M(M-H^+)$ = 175) and the internal standard ($M(M-H^+)$ = 103) based on transitions of *m*/*z* 175→115, *m*/*z* 175→87 and *m*/*z* 103→77. For the optimum MS/MS performance the measuring parameters were: ion spray voltage: -4500 V, ion source temperature: 200°C, declustering potential: -60 V, entrance potential: -10 V, collision energy -24 V. Twenty mL sample was injected in infusion mode. The eluent was water : acetonitrile mixture (80 : 20, v/v) with flow rate of 200 μL/min.

Examination of the stability of L-alanyl-L-glutamine

The stability assay took 14 days at three temperatures (2-8°C, 25°C, 30°C). The measurement was acc. to the internal standard method with glycyl-glycine internal standard. The L-alanyl-L-glutamine was diluted in deionized water to obtain solutions at appropriate concentrations for implementation. The ESI TSQ Quantum Discovery triple quadrupole mass spectrometer equipped with Jasco X-LC binary pump and Jasco X-LC autosampler was used. The ESI-MS/MS system was used in positive ion mode, the quantification of the L-alanyl-L-glutamine ($M(M+H^+)$ = 218) and the internal standard ($M(M+H^+)$ = 133) was based on transitions of *m*/*z* 218→84, *m*/*z* 218→130, *m*/*z* 218→147 for the first compound and *m*/*z* 133→76, *m*/*z* 133→87 for the second. The MS parameters were: spray voltage: 4000 V, sheat gas pressure: 35 psi, aux gas pressure: 5 psi, capillary temperature: 300°C, collision pressure: 1.0 mTorr, scan time: 0.3 s, tube lens offset: 70 V.

Microbiological examinations

The parenteral formula was studied at three different temperatures (2-8°C, 25°C, 30°C) for 14

consecutive days. Aerobic bacterial and mycological cultures were prepared according to the pharmacopoeia monographs.

The samples were treated and evaluated according to the rules of microbiological sample processes. Ten mL of tested TPN samples were inoculated onto Columbia agar containing 5% sheep blood, onto chocolate agar containing polyviteX and onto fungal Sabouraud medium (BioMérieux plates). They were incubated at 37°C for 24 h, then stored at room temperature for another 24 h. Reading and evaluation of the discs were carried out after 24 and 48 h. One mL of the TPN solution was inoculated onto medium, which contains hemin and vitamin K3 thioglycolate, and incubated at 37°C for 24 h. The enriched sample was processed and evaluated in the same manner of the direct blanking.

RESULTS

The most important condition of the nutrition is that the foods should be digested and the nutrients in the digestive system can convert into small molecules. A suitable zeta potential (negative electrical charge) is required, i.e., the nutrients

Table 2. The results of the particle size analysis at different storage temperatures.

Storage time /days	Storage temperature		
	2-8°C	25°C	30°C
	Particle sizes (nm) and relative intensity values		
0	694.9	694.9	694.9
3	497.1	499.2	517.9
4	609.4 – 95.3% 2537 – 4.7%	698.2 – 95.3% 2532 – 4.7%	508.7
5	583.2 – 94.5% 4213 – 5.5%	588.1 – 97.3% 4012 – 2.7%	543.1
6	361.4 – 93.4% 4513 – 6.6%	605.4 – 94.9% 4773 – 5.1%	412.6 – 96.5% 4044 – 3.5%
7	484.6 – 97.6% 5048 – 2.4%	634.3 – 98.4% 4981 – 1.6%	547.5 – 95.2% 5012 – 4.8%
10	497.2 – 97.1% 5205 – 2.9%	698.0 – 97.9% 4997 -2.1%	633.6 – 95.6% 5071 – 4.4%
11	496.0 – 95% 5195 – 5%	655.5 – 96% 5022 – 4%	606.7 – 94.9% 5077 – 5.1%
12	502.5 – 94.5% 5198 – 5.5%	611.3 – 95.6% 5028 – 4.4%	598.8 – 94.2% 5089 – 5.8%
13	499 – 95.2% 5144 – 4.8%	627.2 – 94.8% 5042 – 5.2%	601.3 – 94.2% 5097 – 5.8%
14	400.2 – 94.8% 5187 – 5.2%	624.7 – 93.8% 5087 – 6.2%	596.4 – 93.6% 5100 – 6.4%

Table 3. Results of the two-sample t-test of admixtures stored at different temperatures.

Zeta-potential measurements		
Between 2-8°C and 25°C	p = 0.00071	0.071%
Between 2-8°C and 30°C	p = 0.0017	0.17%
Particle size measurements		
Between 2-8°C and 25°C	p = 0.00071	0.071%
Between 2-8°°C and 30°C	p = 0.0462	4.62%

p refers to the comparison of the zeta-potential and average particle size values with the corresponding values at 2-8°C.

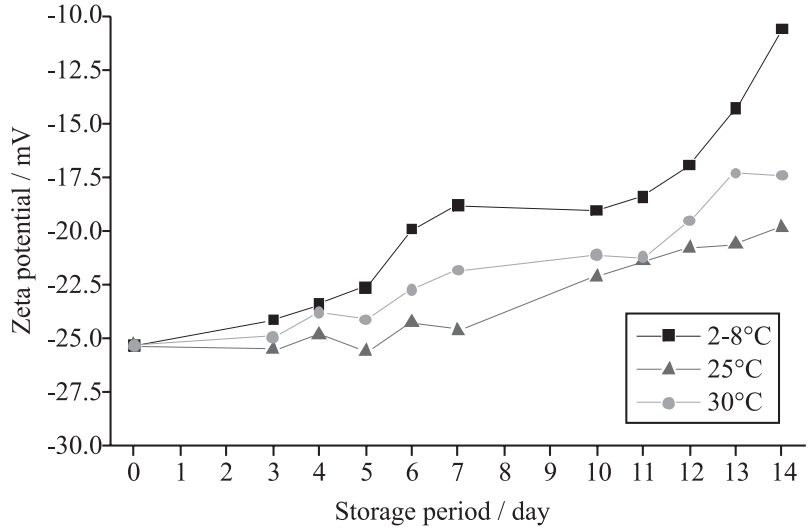

Figure 1. Zeta potential values measured at different storage temperatures as a function of storage time

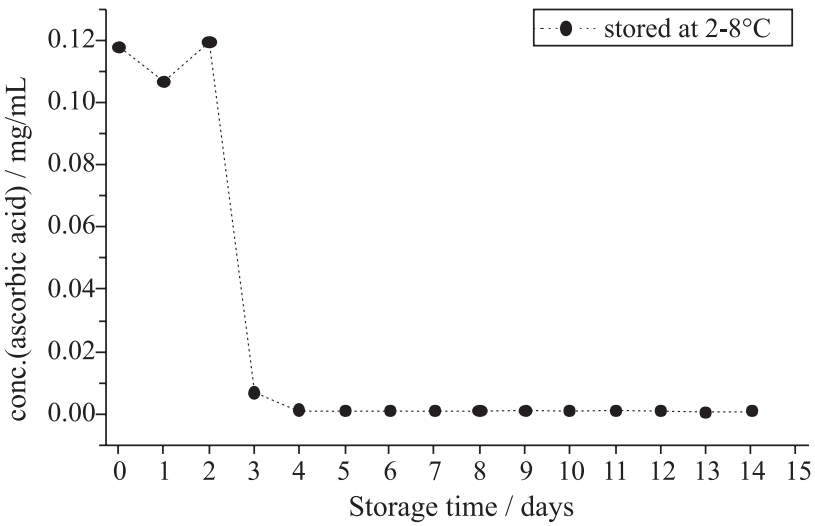

Figure 2. Changes in the concentration of ascorbic acid in the samples stored at 2-8ºC

have to get easily into the cells and to leave from there. As long as the negatively charged nutrients and positively charged intracellular fluid are maintained in equilibrium, the cell metabolism is proper and the way you feel is good. Figure 1 illustrates that the negative zeta potential remained under each storage temperature in the whole 14-day-storage period.

If a patient is unable to be nourished, the parenteral infusion is lifesaver, but efforts should be made to induce fewer side effects with it. It is particularly important that the fat emulsion must have smaller particle size. Under each storage conditions the particle size of the admixtures not even come close to 10 microns during 14 days long study. Table 2 summarizes the particle size distributions of samples stored under different temperatures. The larger particles, which were present in average in 4.6%, did not exceed an average size of 5 microns long red blood cells.

DISCUSSION AND CONCLUSION

There were no significant differences in the average particle size and zeta potential values of admixtures depending on the storage temperatures (Table 3). After examination of the samples, the storage time exceeded even 14 days at all three

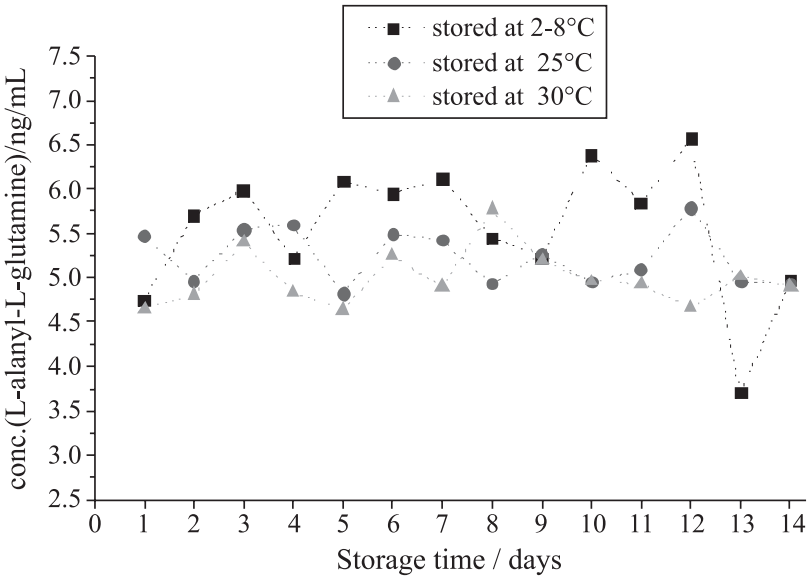

Figure 3. Changes in the concentration of L-alanyl-L-glutamine at different temperatures as a function of storage time

temperatures. Moreover, the results show that the values were the best at 30°C; the 4044 nm particle appeared in 3.5% only on the 6th day. However, it was not possible to detect ascorbic acid at 25°C and 30°C after 24 h. Samples, which were stored at 2-8°C, contained ascorbic acid after 48 h, but after 3 days, the ascorbic acid became immeasurable, hence completely decomposed in each sample (Fig. 2). In contrast to the ascorbic acid, L-alanyl-L-glutamine can be stored for 14 days without decomposition at each of the examined temperature. Figure 3 confirms that the concentration changes of L-alanyl-L-glutamine remained within the acceptable limits. The relative standard deviations (% RSD) are under 10% (6.3 - 9.5%). Each sample remained sterile within the whole storage interval.

According to the results of the physicochemical examinations, 10-day storage period of this type of TPN admixtures at room temperature can be accepted. Their storage does not require refrigeration (2-8°C), thus they can be administered without special preheating ensuring better physiological tolerance. Because of the rapid decomposition of vitamin C, the water-soluble Soluvit multivitamin has to be added into the admixture in every day, while the glutamine can be mixed with the amino acid infusion because of its adequate stability.

REFERENCES

1. Skouroliakou M., Matthaiou C., Chiou A., Panagiotakos D., Gounaris A. et al.: Eur. J. Hosp. Pharm. 19, 514 (2012).
2. Bouchoud L., Sadeghipour F., Klingmuller M., Fonzo-Christe C., Bonnabry P.: Clin. Nutr. 29, 808 (2010).
3. Meyer R., Timmermann M., Schulzke S., Kiss C., Sidler M.A., Furlano R.I.: Nutrients 5, 2006 (2013).
4. Balogh J., Kiss D., Dredan J., Puskas I., Csempesz F., Zelko R.: AAPS PharmSciTech 7, 98 (2006).
5. Silvers K.M., Sluis K.B., Darlow B.A., McGill F., Stocker R., Winterbourn C.C.: Acta Pediatr. 90, 242 (2001).
6. Silvers K.M., Darlow B.A., Winterbourn C.C.: J. Parenter. Enteral. Nutr. 25, 14 (2001).
7. Bouchoud L., Sadeghipour F., Fonzo-Christe C., Pfister R., Bonnabry P.: P312, 30th ESPEN Congress, Florence, 2008.
8. Skouroliakou M., Matthaiou C., Chiou A., Panagiotakos D., Gounaris A. et al.: J. Parenter. Enteral Nutr. 32, 201 (2008).
9. Telessy I.G., Balogh J., Szabo B., Csempesz F., Zelko R.: Nutr. J. 11, 33 (2012).
10. Telessy I.G., Balogh J., Csempesz F., Szente V., Dredan J., Zelko R.: Colloids Surf. B Biointerfaces 72, 75 (2009).

11. Vasques R., Le Hoang M.-D., Martin J., Yahia Y.A., Graffard H. et al.: Eur. J. Hosp. Pharm. Sci. 15, 28 (2009).
12. Ivanovic D., Popovic A., Radulovic D., Medenica M.: J. Pharm. Biomed. Anal. 18, 999, (1999).
13. Fenoll J., Martínez A., Hellín P., Flores P.: Food Chem. 127, 340 (2011).
14. Koletzko B., Goulet O., Hunt J., Krohn K., Shamir R., Parenteral Nutrition Guidelines Working Group; European Society for Clinical Nutrition and Metabolism; European Society of Paediatric Gastroeneterology, Hepatology and Nutrition (ESPGHAN); European Society of Paediatric Research (ESPR).: J. Pediatr. Gastroenterol. Nutr. 41, S2, S1-87, (2005).
15. Heudi O., Kilinc T., Fontannaz P.: J. Chromatogr. A 1070, 49 (2005).
16. Klek S., Chambrier C., Singer P., Rubin M., Bowling T. et al.: Clin. Nutr. 32, 224 (2013).

Received: 16. 06. 2014