Long-term physico-chemical stability of standard parenteral nutritions for neonates

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SUMMARY

Background & aims: Two ready-to-use parenteral nutritions (PN) have been developed, for the first days of life of the premature newborn, along with syringes of lipid emulsion with or without vitamins. Long-term physico-chemical stability for storage in wards was assessed.

Methods: Physico-chemical stability of PN: visual inspection, particle size, pH, osmolarity measurement, amino acids, glucose, and electrolytes dosages. Physico-chemical stability of lipid emulsion: visual inspection, globule size, peroxide level and vitamins A, E, and C dosages. Stability was studied for 12 weeks on refrigerated (2–8 °C) and room temperature (30 ± 2 °C) samples.

Results: No precipitation was detected in any PN. A brown coloration was observed in PN stored for four weeks at room temperature but not in the refrigerator. Concentrations of all the nutrients remained constant over the 12 week-study period. Phase separation of the lipid emulsion occurred after three weeks, but particle size complied with the USP limits for 12 weeks. Peroxide content increased only in the samples without vitamins at room temperature. Vitamins remained stable for one week under refrigeration.

Conclusion: The PN did not present a detectable change of the tested properties when refrigerated for 12 weeks. The lipid emulsion with vitamins is stable for one week when refrigerated.

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1. Introduction

Providing ready-to-use parenteral nutrition (PN) in a single container is a safe, economic and ergonomic way of delivering it. Due to the multi-composition nature of PN, its stability can be easily disturbed by physicochemical factors. Instability can result in the formation of precipitates, chemical reactions of components as well as degradation of nutrient concentration, and emulsion coalescence. Lipid emulsions are thermodynamically unstable and the two phases tend to separate over time. A visible phase separation can be reversible when the emulsion is agitated if there has not happened a coalescence of the lipid globules, whereas growth of the lipid droplets into large fat globules (>5 μm) is irreversible, and could be dangerous if administered. This instability is mainly favoured by low pH, high concentration of positively-charged ions, and trace elements. In the presence of oxygen, UV-light or high temperature, lipids can be oxidised and can form lipid peroxides being converted to reactive species as free radicals. A few studies in humans, especially in preterm newborns, assessed the extent of peroxidation in parenteral fat emulsions and showed the potential toxic risk of degraded components in vivo. Peroxidation could possibly be responsible for the development of complications typically occurring in premature infants, including bronchopulmonary dysplasia and retinopathy. To avoid such degradation some authors recommend administering vitamins in lipid emulsion, to benefit from the anti-oxidative activity of vitamin C and E. Beside the deterioration of the lipid emulsion, several additional physical...
and chemical effects can occur. Amino acids and glucose can interact together forming Maillard reaction products. This chemical reaction appears when an amine function (such as the amine function from amino acids) and a reducing sugar (as glucose) react together and form a variety of brown-coloured products. Precipitation of calcium phosphate can also happen, but can be overcome by using organic salts. Trace elements are catalytic in a majority of oxidation reactions, and are best added to PN just before administration. Vitamins are the less stable elements of PN, particularly vitamin C, which oxidises very quickly when oxygen is present. To combat this problem, they too need to be added just prior to administration and a light protection system is used during infusion (via a dark infusion tube) to limit loss of light sensitive vitamins. To negate all the physicochemical problems encountered with ternary PN, commercialized PN are packaged in two or three-compartment bags allowing the separation of amino acids, glucose and lipid emulsion until the time of administration. In hospitals, individual PN's are generally prepared in one compartment bag and the pharmacist is responsible for guaranteeing its stability until the end of the administration.

In our hospital, the pharmacy is responsible for providing PN to pediatric patients, particularly during the first days of life of the premature newborns. As no PN are registered in Switzerland for this population, tailored-made PN are compounded daily. A retrospective study on PN prescriptions revealed that few premature newborns benefit from PN since their first day of life (internal data). Generally, for organisational reasons, they receive only glucose for one to three days, and need to wait to receive the appropriate PN. This creates a lack of amino acid supplies for these infants. To improve the nutritional care of premature newborns in our institution, we decided to provide ready-to-use standard (STD) PN which can be stored in the wards and administered when needed. In cooperation with the neonatologist, two formulae of STD PN were formulated, containing a high concentration of amino acids, in accordance with ESPGHAN/ESPEN guidelines. The first formula without electrolytes was designed for the day of birth (D0) of premature newborns (amino acids 3%, glucose 10.8% and heparin 0.5 UI/mL), and the second one for the following four days (D1-4) (amino acids 3%, glucose 10.8%, Na 20 mmol/L, K 10 mmol/L, Ca 11 mmol/L, PO4 8.6 mmol/L and heparin 0.5 UI/mL). As the stability of the lipid emulsion is the most critical point for long shelf-life, we decided to provide individual syringes of lipid emulsion (including vitamins or not) apart from STD PN. The volumes of PN administered are dependent on the premature newborns and they need to be added just prior to administration and a light protection system is used during infusion to negate all the physicochemical problems encountered with ternary PN, commercialized PN are packaged in two or three-compartment bags allowing the separation of amino acids, glucose and lipid emulsion until the time of administration. In hospitals, individual PN's are generally prepared in one compartment bag and the pharmacist is responsible for guaranteeing its stability until the end of the administration.

The aim of this study is to evaluate the physicochemical stability of both STD PN formulae as well as the lipid emulsion, with or without vitamins, over a period of 12 weeks.

2. Material and methods

2.1. Preparation of PN admixture

Both STD PN and lipid emulsion syringes were aseptically compounded at the pharmacy in a horizontal laminar airflow hood (ISO 4.8 or Grade A GMP) located in a grade B GMP (ISO 5) clean room. PN were prepared in two different volumes. PN of 150 mL were prepared in a multilayered (ML) plastic bag (Nutripoche S71, Stedin, France) and 60 mL PN into polypropylene (PP) syringes (Plastipack, Becton-Dickinson, England) using an automated filling system: Baxa MicroMacro 12 (Baxa Corporation, Englewood, CO, USA). Lipid emulsions (with or without vitamins) were filled into PP syringes (10 and 20 mL) (Plastipack, Becton-Dickinson, England). The composition and presentation of STD PN and lipid syringes are presented in Tables 1 and 2, respectively.

2.2. Storage

STD PN admixtures and syringes of the lipid emulsion were protected from light and stored at two different temperatures: in an incubator between 30 ± 2 °C corresponding to the room temperature (RT) in neonatal wards, and in the refrigerator (F), between 2 °C and 8 °C.

2.3. Study protocol

2.3.1. STD PN

Physical stability was assessed by visual inspection and by measuring particle contamination, pH and osmolarity. Chemical stability was evaluated by the quantitative analyses of amino acids, glucose, and electrolytes (calcium, potassium, sodium, phosphate). Two samples of both STD PN (D0 and D1-4), in each container (syringe and bag) at both temperatures were analysed at starting time (just after mixing) and after 1, 2, 3, 4, 6, 8 and 12 weeks. Results are expressed as the average value of the results obtained from the two parallel samples.

2.3.2. Lipid emulsion syringes

Droplet size distribution and peroxide content were assessed at commencement (just after filling) and later, after 1, 2, 3, 4, 6, 8 and 12 weeks. Vitamin stability was assessed by quantitatively determining vitamin A, C and E levels at the same time intervals, until the concentration of one ingredient fell below 90% of the initial concentration. Two samples of lipid emulsion without vitamins and two samples with vitamins were analysed at both temperatures.

3. Analyses of STD PN

3.1. Physical stability

Samples were visually inspected using the European Pharmacopeia (EP) visual inspection conditions against a black and white contrast background for signs of precipitation. Particle size was assessed in clear samples as recommended by EP (chapter 2.9.19) with a Hiac/Royco 90/64 laser light extinction particle counter

Table 1

<table>
<thead>
<tr>
<th>Components of STD PN</th>
<th>D0 syringe, 60 mL</th>
<th>D0 bag, 150 mL</th>
<th>D1-4 syringe, 60 mL</th>
<th>D1-4 bag, 150 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 70% (mL)</td>
<td>9.3</td>
<td>23.3</td>
<td>9.3</td>
<td>23.3</td>
</tr>
<tr>
<td>Amino acids (mL)</td>
<td>27.6</td>
<td>69</td>
<td>27.6</td>
<td>69</td>
</tr>
<tr>
<td>NaCl 11.7% (mL)</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>KCl 7.5% (mL)</td>
<td>0</td>
<td>0.6</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca gluconate 10% (mL)</td>
<td>0</td>
<td>4.1</td>
<td>0</td>
<td>10.3</td>
</tr>
<tr>
<td>PO42- (mL)</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Heparin 50 UI/mL</td>
<td>0.6</td>
<td>1.5</td>
<td>0.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

# References

1. Fresenius Kabi, Germany.
2. Vaminolact® (Fresenius Kabi, Switzerland).
3. Bichsel AG, Switzerland.
4. Phocytan® (glucose-1-phosphate, Aguettant, France).
5. Internal production, HUG pharmacy, Switzerland.
4. Analyses of lipid emulsion syringes

4.1. Physical stability

All the samples were visually inspected throughout the study for evidence of phase separation.

The mean droplet size (MDS) diameter was determined by using dynamic light scattering (Nicomp 380, Particle Sizing Systems, Santa Barbara, CA, USA). Samples have been prediluted 1:10 with filtered water. Additional automatic dilution to a photopulse rate of 250–350 kHz was done using the test system. Mean lipid droplet size was calculated, based on the intensity-weighted data.

Light obscuration was used to assess the large-diameter tail of droplet size distribution (AccuSizer 780 APS, Particle Sizing Systems, Santa Barbara, CA, USA), after automatic dilution of the sample with filtered water. Overall, the dilution of the sample was 1:1800.

To be considered stable, MDS must not exceed 500 nm and the volume-weight percentage of fat > 5 μm (PFAT5) must not exceed 0.05%, as recommended by the USP.

4.2. Chemical stability

 Peroxide concentration was measured by spectrophotometry at 560 nm by the FOX method as described by Gräfein et al. Results are expressed as mmol of tert-butyl hydroperoxide (TBH) equivalent/L. No limit of acceptation could be determined as no specifications are listed in any pharmacopoeia. Concentrations of vitamins A and E were measured together by HPLC with UV detection, following the method described by Dupertuis et al. and vitamin C by HPLC with amperometric detection. The stability of vitamins was determined by a final concentration of more than 90% of the initial concentration.

5. Results

5.1. STD PN

5.1.1. Physical stability

Refrigerated STD PN remained clear throughout the study period, whereas a brown coloration appeared in the STD PN stored at RT, after four weeks of storage. No precipitate was identified in any STD PN. Particle count remained within the EP specifications for all the samples. The formula D0 presented a pH of 5.1 and an osmolality of 950 mosm/L. The formula D1-4 presented a pH of 5.8 and an osmolality of 1000 mosm/L.

5.1.2. Chemical stability

No significant loss was determined in any sample (bags or syringe, F or RT, D0 or D1-4) of any nutrient tested. Amino acids, glucose and electrolytes concentrations did not fall below 90% of the initial concentration, throughout the study period as illustrated in Figs. 1 and 2 for amino acids and glucose. Heparin activity hovers between 80 and 110% of the targeted activity.

5.2. Lipid emulsion syringes

5.2.1. Physical stability

Phase separation was visually observed in lipid syringes since the third week of storage and increased until the end of the study. No significant changes occurred in the mean diameter of fat globules or in the PFAT5 throughout the study period, and these parameters complied with the USP limits for 12 weeks (Table 3).
5.2.2. Chemical stability

Peroxide concentration in the lipid emulsion without vitamins rose from 0.21 to 2.1 mmol/L of TBH equivalent when stored at RT. The peroxide concentration increased slightly over the storage period in refrigerated samples or containing vitamins, as presented in Fig. 3.

Vitamin C concentration fell below 90% after two weeks of storage. Vitamin A and E concentrations remained above 90% after two weeks storage. The study was then interrupted after two weeks due to vitamin C instability (Table 4).

6. Discussion

The objective of developing STD PN was to ensure an appropriate nutritional support to neonates since the first day of life, by providing safe ready-to-use compounded products. A high concentration of amino acids was chosen to increase the amount of proteins administered during the first days of life of premature newborns, as recommended by ESPGHAN/ESPEN guidelines.9

Our study confirmed the stability of the formulations developed, as we observed not more than 10% loss of any nutrient, irrespective of the formula, container, or temperature, over 12 weeks of storage. Significant coloration appeared in STD PN stored at RT since the fourth week of storage, which was probably due to Maillard reaction. Reaction rate is temperature dependent,16 explaining the lack of coloration in the refrigerated STD PN. Quantitative analyses did not show any significant decrease in glucose or amino acid concentrations; however, the administration of coloured solutions is not recommended.

No loss or change of electrolytes was noted throughout the study. Calcium and phosphate concentrations remained stable during the study period with no increase in particulate matter, indicating an absence of calcium phosphate precipitation.

STD PN stability is optimal when they are stored in the refrigerator. No coloration appeared at this temperature and all parameters remained stable. However, based on the data presented, STD PN can be stored for a few weeks at RT. This indication can be useful when the PN bags are neglected outside the refrigerator or when the refrigerator breaks down. Under such conditions, our data guaranteed the quality of STD PN stored at RT up to three weeks.

Only limited data have been published regarding the long-term stability of the binary PN. Most studies focused on the lipid emulsion stability in ternary STD PN,17−21 because lipids were suspected to be the most sensitive component of total PN. For a long storage period, the stability of all nutrients must be ensured. A study showed that amino acids in the refrigerated PN solution (glucose, amino acids, electrolytes and trace elements) stored in PVC bags decreases in concentration by more than 10% after two months.22 In our study, we used multilayer bags, to restrict oxygen permeation, and increase the stability of amino acids and other nutrients sensitive to oxygen. Consequently, we found no loss of amino acids after 12 weeks, confirming the efficient protection provided by the multilayers.

Degradation of vitamins can also markedly decrease the shelf-life of a PN. In a stability study of ternary PN, the two PN formulae tested were physically stable up to seven days, although the concentration of vitamin E decreased after 24 h, limiting the period of storage to 1 day.23 These PN were compounded in ethylvinyl acetate (EVA) bags, highly permeable to oxygen. In our study, the syringes used as primary packaging material for the lipid emulsion with or without vitamins, were made of PP (almost impermeable to oxygen) probably resulting in an acceptable stability of the vitamins tested.

Dropet size measurement showed no coalescence of the emulsion, as MDS and PFAT5 remained stable throughout the study period. However, phase separation was visually observed since the third week of storage, and although this is a reversible phenomenon, it is not recommended to deliver such preparations to the wards. For safety reasons, we decided to limit the shelf-life of the lipid emulsions to two weeks.

Table 3
Physical stability of lipid emulsion (protected from light).

<table>
<thead>
<tr>
<th>Time (week)</th>
<th>Lipids without vitamins</th>
<th>Lipids with vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Storage at RT</td>
<td>Storage in the F</td>
</tr>
<tr>
<td></td>
<td>MDS (mm)</td>
<td>PFAT5 (%)</td>
</tr>
<tr>
<td>0</td>
<td>281 0.01</td>
<td>282 0.01</td>
</tr>
<tr>
<td>1</td>
<td>276 0.02</td>
<td>277 0.015</td>
</tr>
<tr>
<td>2</td>
<td>278 0.01</td>
<td>279 0.02</td>
</tr>
<tr>
<td>3</td>
<td>285 0.01</td>
<td>280 0.01</td>
</tr>
<tr>
<td>4</td>
<td>276 0.01</td>
<td>280 0.01</td>
</tr>
<tr>
<td>6</td>
<td>269 0.01</td>
<td>272 0.02</td>
</tr>
<tr>
<td>8</td>
<td>277 0.01</td>
<td>277 0.02</td>
</tr>
<tr>
<td>12</td>
<td>270 0.01</td>
<td>276 0.01</td>
</tr>
</tbody>
</table>

PFAT5: percentage of fat globule > 5 μm related to the total lipid volume, MDS: mean size droplet diameter, RT: room temperature, F: fridge.

Table 4
Stability of vitamins in lipid emulsion (protected from light).

<table>
<thead>
<tr>
<th>Time (week)</th>
<th>Lipid emulsion stored at RT</th>
<th>Lipid emulsion stored F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamins</td>
<td>Vitamins</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>E</td>
</tr>
<tr>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>1</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>96%</td>
<td>102%</td>
</tr>
<tr>
<td>3</td>
<td>277%</td>
<td>79%</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>47%</td>
</tr>
</tbody>
</table>
At commencement, the hydroperoxide content of the samples with vitamins was higher than those without vitamins. The reason of this observation may be an interaction of the applied FOX test with vitamins.

No significant increased level of peroxide equivalents occurred in the refrigerated emulsion samples with or without vitamins. The emulsion used in this study is a mix of medium- and long-chain triglycerides (MCT/LCT). MCT/LCT emulsions contain much fewer polyunsaturated fatty acids that are at high-risk of peroxidising compared with emulsions containing only LCT (31% vs 61%), decreasing the risk of peroxidation. The quantities of peroxide equivalents in samples without vitamins stored at RT increased by 10 times over the 12 weeks storage period, illustrating both the impact of the temperature on the reaction, and the protective role played by vitamins, as observed by other authors.7

Vitamin degradation was limited in the emulsion. More than 80% of the initial value of vitamin C content was still present after two weeks of storage. In a study evaluating the vitamin stability in the refrigerated PN stored in EVA bags, the authors measured only around 20% of the initial vitamin C concentration after 24 h.14 Lipid emulsion and containers like a syringe in PP, which are quite impermeable to oxygen, protect vitamins from oxidation. Several studies encourage the use of lipid emulsion with vitamins at 12 weeks and at one week, respectively, illustrating both the reason of this observation may be an interaction of the applied FOX test with vitamins.

The heparin activity measured in this study had not been performed applying an appropriate method. Consequently, the results obtained are only semi-quantitative, although they show that there is a detectable activity even after 12 weeks of storage.

Based on our data, we fixed the shelf life for STD PN and lipid emulsion with vitamins at 12 weeks and at one week, respectively, under refrigeration. This is sufficient for batch production and to allow storage of STD PN and lipid emulsion syringes directly in the wards. This logistic organisation guarantees the possibility of using them as soon as required, without any delay, which is definitely a major advantage over tailor-made PN.

These STD PN are available in the wards since February 2009. It would be therefore interesting to evaluate the clinical impact of administering these STD PN, very rich in amino acids, since the day of birth of premature newborns, compared with the normal practice with glucose and tailored PN.

Conflict of interest

The study was supported by a financial grant from B/Braun.

Acknowledgement

Statement of authorship: LB was involved from the conception of the study, performed the physical stability analyses on STD PN as vitamin dosage and drafted the manuscript; CFC and FS were involved in designing the study, in interpreting the data and producing the manuscript. MK conducted the analyses, mostly on lipid emulsion and chemical analyses on STD PN. PB supervised the design and the execution of the study, and contributed toward writing the manuscript. All authors read and approved the final version of the manuscript.

References