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Assessment of the Physicochemical Stability of All-in-One Parenteral Emulsions for Neonates According to USP Specifications

Christos Athanasiou, MD1; Sophia Hatziantoniou, PhD1; Maria Skouroliakou, PhD2; and Sophia Markantonis-Kyroudis, PhD1

Abstract

Background: The purpose of this study was to describe the methodology to assess the stability of all-in-one (AIO) parenteral nutrition admixtures, containing glucose, proteins, and lipids, to the standards of U.S. Pharmacopoeia (USP <729>). The influence of calcium and commercially available lipid emulsions and amino acid solutions were also examined. Methods: Four batches of 5 AIO admixtures containing calcium were compounded with commercially available lipid emulsions and amino acid solutions. Two of them contained calcium. Their stability was tested under conditions simulating clinical use. All the admixtures were assessed for criteria set by the USP <729>: (1) mean droplet diameter (MDD) and (2) percentage of volume weighted particles with diameter > 5 μm (PFAT5). Results: All admixtures were within the specifications set by the USP with respect to the MDD at 0, 24, and 48 hours, but only those batches lacking calcium met the benchmarks set by the pharmacopoeia, with respect to PFAT5, on the day of preparation. Conclusions: The presence of calcium destabilized the admixtures, while the use of different commercial ingredients altered the admixtures’ characteristics. Only 1 batch of the AIO admixtures studied was found to be compliant with USP <729> standards. (JPEN J Parenter Enteral Nutr. XXXX;xx:xx-xx)

Keywords

all-in-one parenteral emulsion; neonates; physicochemical stability, emulsions

Clinical Relevancy

All-in-one (AIO) admixtures for parenteral nutrition for neonates prepared using different commercial ingredients, with or without the presence of calcium, in conditions simulating clinical use. Their adherence with U.S. Pharmacopoeia (USP) <729> standards was tested. The findings of the study may be used as a guide for the assessment of AIO admixtures for neonates.

Introduction

Parenteral nutrition (PN) aims to provide the patient with all the essential macro and micronutrients, including amino acids, carbohydrates, lipids and electrolytes required daily. It has been argued that the use of PN admixtures with all the nutrients in the same container, reduces manipulation during administration and the risk of contamination, and may be more cost-effective. However, such AIO admixtures are extremely complex and inherently unstable. Due to their numerous ingredients an unequivocal prediction of their stability and safety is impossible. Moreover, FDA has published an alert for the hazards of precipitation associated with PN, since deaths have been attributed to undetected sediment. Calcium phosphate precipitation was a fairly common problem but lately, the use of organic phosphorus has partially solved this issue. Nevertheless every admixture extemporaneously prepared in the hospital pharmacy must be thoroughly examined for those characteristics considered essential for their safety, for example, the size distribution of the lipid droplets, for which the USP has set certain limits. AIO admixtures for neonates are not in use since the restricted volume of fluids permitted for administration in combination with the high needs of neonates for electrolytes make these admixtures extremely concentrated and therefore unstable. However, according to studies conducted lately, AIO admixtures based on Medium and Long Chain Triglycerides (MCT/LCT) emulsions, like Smooflipid® 20% used in this study are considered more stable.
The aim of this study was to describe the methodology used to assess the stability of a series of 5 AIO admixtures, for neonates with gestational age of 26-28 weeks, to the standards set by the USP (USP <729>). The influence of different commercially available ingredients and the presence of calcium ions on their stability were also examined. The AIO admixtures were prepared at the maternity hospital IASO, Athens.

Clinical Relevancy Statement

Although the use of AIO admixtures for PN in adult patients is already common practice, preparing admixtures for neonates is still problematic due to stability and compatibility issues. Overall, preparing AIO bags for PN in neonates may benefit everyday clinical practice and possibly reduce costs. With a view to producing AIO admixtures that will meet neonates’ special nutrition needs, we have studied a series of admixtures prepared for the first days of life of a specific subgroup of premature neonates and assessed their compliance with USP <729> standards.

Materials and Methods

Preparation of PN Admixtures

For the preparation of the AIO admixtures the following products where used:

AA1 (amino acid solution); Vamin Infant (Fresenius Kabi Hellas Sa): An electrolyte-free solution for intravenous (IV) nutrition that provides a mixture of essential and nonessential amino acids as well as cysteine, tyrosine, histidine. The total concentration of the amino acids is 65.3 g/L, essential amino acids 3.9 g, and pH 5.2.

LE1 (lipid emulsion) Smoflipid® 20 (Fresenius Kabi Hellas Sa): An α-tocopherol-enriched lipid emulsion consisting of soybean oil as the source of essential fatty acids (30%), medium-chain triglycerides, rapidly oxidized to provide energy (30%), refined olive oil as the source of monounsaturated fatty acids, in particular oleic acid (25%), and fish oil as the source of very long-chain ω-3 fatty acids (15%). pH approximately 8.

LE2 (lipid emulsion) ClinOleic® 20% (Baxter Hellas Ltd): A sterile fat emulsion containing of a mixture of refined olive oil (approximately 80%) and refined soya oil (approximately 20%). It also contains egg lecithin (purified egg phospholipids), glycerol, sodium oleate, and water for injections. One of the active ingredients, soya oil, contains ascorbyl palmitate as an antioxidant (0.15 mg per g of oil). pH range 6.0-8.0.

Table 1. Composition by Volume (ml) of Batches C1 and S1 AIO Admixtures.

<table>
<thead>
<tr>
<th></th>
<th>AI02</th>
<th>AI03</th>
<th>AI04</th>
<th>AI05</th>
<th>AI06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid solutiona</td>
<td>48.2</td>
<td>56.4</td>
<td>64.5</td>
<td>85.4</td>
<td>72.1</td>
</tr>
<tr>
<td>Dextrose 50%</td>
<td>28.8</td>
<td>34.2</td>
<td>42</td>
<td>52.8</td>
<td>46.9</td>
</tr>
<tr>
<td>Lipid emulsionb</td>
<td>11.0</td>
<td>13.5</td>
<td>16</td>
<td>16.8</td>
<td>19.5</td>
</tr>
<tr>
<td>KCl 10%</td>
<td>1.9</td>
<td>1.8</td>
<td>1.8</td>
<td>2.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Ca gluconate10%c</td>
<td>5.6</td>
<td>7.0</td>
<td>8.2</td>
<td>11.1</td>
<td>9.4</td>
</tr>
<tr>
<td>MgSO4.7H2O 25%</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium glycerophosphate anhydrous 21.6% w/vd</td>
<td>0.8</td>
<td>1.1</td>
<td>1.3</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Water soluble vitaminsg</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Lipid soluble vitaminsf</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>6.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Heparin</td>
<td>1.5</td>
<td>1.7</td>
<td>3.6</td>
<td>4.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Water for injection</td>
<td>37.5</td>
<td>35.4</td>
<td>28.8</td>
<td>25</td>
<td>31.6</td>
</tr>
</tbody>
</table>

The synthesis of admixtures AI02-AIO6. Batch C2 and batch S2 were free of calcium. Fluid retention from automatic compounder 25 ml, lipid retention 6 ml.

aAA2 for batch C and AA1 for batch S. These volumes refer to AA1; for AA2 the volumes used were adapted and the difference was replenished with water for injection. The total volume was equal for both batches.

bLE2 for batch C and LE1 for batch S, respectively.

cVolumes refer to batch C1 and batch S1. Batches C2 and S2 were free of calcium.

dGlycophos®, Fresenius Kabi.

eSoluvit®, Fresenius Kabi.

fVitalipid®, Fresenius Kabi.
administered, so no AIO admixture is prepared. All nutrients are calculated per body weight; every admixture fulfills the requirements for 1 kg body weight. To assess the influence of electrolytes on their chemical and physical stability, a separate batch of the 5 AIO admixtures (batch 2) was prepared free of calcium.

As the final stability of AIO compounded admixtures is also known to be affected by the composition of commercial lipid emulsions and commercial solutions of amino acids,15,16 each batch of admixtures (batch 1 and batch 2) was prepared in 2 different ways using the most common commercial formulations in Greece. The first using LE1 as the lipid emulsion in combination with AA1 as the amino acid solution (batch S) and the second using LE2 as the lipid emulsion in combination with AA2 as the amino acid solution (batch C). Overall, 4 batches of 5 AIO admixtures (S1, S2, C1, C2) were prepared and tested.

In our experience, it is much more common and convenient for a hospital’s PN unit to use 1 company’s products at any 1 time. Thus, in this study amino acid solutions and lipid emulsions were not cross-checked; the same combination of both nutrients (essential and semiessential amino acids and fat emulsions) was furnished by each manufacturer.

The admixtures were prepared in duplicate bags made of ethyl vinyl acetate ester, at the IASO maternity hospital, using an automated filling (MicroMacro 12 compounder; Baxa, Englewood, CO).

**Methodology to Assess Stability of Admixtures**

**Visual inspection of samples.** For visual inspection of AIO parenteral admixtures, each bag was inverted for sampling 5 times, to test the redispersability of flocculation or sedimentation. An aliquot of the emulsion was placed in a beaker to observe its surface under normal light. The appearance of large colorless or yellow droplets at the surface was considered to indicate phase separation and unacceptable emulsion stability.

**Physicochemical characterization according to USP <729>**. The USP has set the following limits: (1) the intensity-weighted mean droplet diameter (MDD) for lipid injectable emulsions must be < 500 nm or 0.5 μm, irrespective of the concentration of the dispersed lipid phase and (2) the volume-weighted, large-diameter fat globule limits of the dispersed phase, expressed as the percentage of fat residing in globules larger than 5 μm (PFAT5) for a given lipid injectable emulsion, must be < 0.05%.

**MDD assessment.** The admixtures were assessed for 2 parameters, namely the MDD and the volume weighted percentage of droplets with diameter > 5 μm (PFAT5). To estimate the average droplet diameter (MDD), as required by the pharmacopoeia, photon correlation spectroscopy (Zetasizer 3000HSA; Malvern, UK) was applied. Of each sample, 16 μl aliquots were diluted with water for injection (WFI) to a final volume of 3 ml. Measurements were performed at 25°C, detection wavelength 633 nm, and a fixed angle of 90°. For data analysis the Contin method (Malvern Software) was used.

**PFAT, assessment.** To estimate the “tail of large diameter,” laser diffraction was applied (Mastersizer S, Malvern Instruments Ltd, Malvern, UK; fitted with a small volume sampler set at 50% of its speed capacity). A 330 mm size focal lens was used with detection range 0.5-880 μm. The Real Refractive Index was set to 1.456 and the Imaginary Refractive Index to 0.01. The background signal was adjusted after filling the stirrer with WFI. A small quantity of each admixture was removed, using a plastic syringe, and added drop-wise into the stirrer until obscuration reached 14%. For each admixture the measurement was repeated twice.

**Simulation of Clinical Use Conditions**

To assess the admixtures under simulated clinical conditions the AIO bags were prepared in duplicate. One of each pair of bags was used for the assessment of the size distribution of the droplets within 3 hours of preparation and then connected to a supply pump (module MPV, Fresenius Kabi) and reassessed at 24 hours. The admixture flow rate was set at 2 ml/h. The second bag was stored at 4°C for 24 hours and assessed before (24 hours at 4°C) and after its connection to a supply pump for a further 24 hours of pumping (48 hours after preparation).

**Results**

**Visual Inspection of Samples**

On the day of preparation all samples were subjected to optical observation for detection of signs of instability such as creaming, flocculation, and sedimentation. We observed the following:

1. Batches S1 and C1: Within 24 hours of their preparation, creaming was noted. For some of these admixtures creaming was not easily reversed with gentle shaking, indicating the beginning of coalescence that is an irreversible stage of instability.
2. Batches S1 and C1: Signs of precipitation were observed within a few hours of their preparation.
3. Batches S2 and C2: Optical observation did not reveal any sediment or creaming.

Of the 4 batches of AIO admixtures assessed, only S2 and C2 (ie, all 5 samples of the batch) were entirely within USP <729> limits on the day of preparation. Moreover, batch C2 remained stable and within specifications after 24 hours at room temperature and at 4°C.
Physicochemical Characterization According to USP <729>

**MDD assessment.** All admixtures assessed (S1, S2, C1, C2 batches of AIO2-AIO6) were within the specifications set by U.S. Pharmacopoeia. Table 2 shows the results for each of the twenty admixtures on the preparation day. For all admixtures and both storage conditions at each time point, this parameter was within specifications.

**PFAT5 assessment.** Only 2 batches, both lacking calcium, were in accordance with benchmarks set by the pharmacopoeia on the day of preparation. In addition, only 1 of them (batch C2) remained stable and within specifications for 24 hours at both room temperature and at 4°C. Detailed results for each admixture are presented in Tables 3 and 4.

### Table 2. Droplet Size Distribution of AIO Admixtures (MDD) on the Preparation Day (nm).

<table>
<thead>
<tr>
<th></th>
<th>S1&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th>S2</th>
<th></th>
<th></th>
<th>C1&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDD ± SD</td>
<td>PI&lt;sup&gt;d&lt;/sup&gt;</td>
<td>MDD ± SD</td>
<td>PI</td>
<td>MDD ± SD</td>
<td>PI</td>
<td>MDD ± SD</td>
<td>PI</td>
</tr>
<tr>
<td>AIO2</td>
<td>300.2 ± 4.2</td>
<td>0.055</td>
<td>297.4 ± 4.0</td>
<td>0.072</td>
<td>262.9 ± 7.3</td>
<td>0.042</td>
<td>271.9 ± 0.9</td>
<td>0.057</td>
</tr>
<tr>
<td>AIO3</td>
<td>300.1 ± 2.7</td>
<td>0.067</td>
<td>298.2 ± 1.4</td>
<td>0.028</td>
<td>263.1 ± 8.4</td>
<td>0.037</td>
<td>270.7 ± 2.3</td>
<td>0.047</td>
</tr>
<tr>
<td>AIO4</td>
<td>305.3 ± 2.7</td>
<td>0.052</td>
<td>299.1 ± 2.7</td>
<td>0.064</td>
<td>267.4 ± 5.1</td>
<td>0.054</td>
<td>273.6 ± 3.7</td>
<td>0.030</td>
</tr>
<tr>
<td>AIO5</td>
<td>298.9 ± 1.5</td>
<td>0.107</td>
<td>291.3 ± 0.7</td>
<td>0.096</td>
<td>265.4 ± 4.4</td>
<td>0.065</td>
<td>268.6 ± 4.8</td>
<td>0.058</td>
</tr>
<tr>
<td>AIO6</td>
<td>298.8 ± 1.4</td>
<td>0.107</td>
<td>291.1 ± 1.1</td>
<td>0.086</td>
<td>263.9 ± 5.7</td>
<td>0.046</td>
<td>272.0 ± 3.1</td>
<td>0.070</td>
</tr>
</tbody>
</table>

The synthesis of admixtures AIO2-AIO6, as presented in Table 1. Batch C2 and batch S2 were free of calcium.

<sup>a</sup>LE1 and AA1 for batch S.
<sup>b</sup>LE2 and AA2 for batch C.
<sup>c</sup>MDD: mean droplet diameter.
<sup>d</sup>Polydispersity index (PI).

### Table 3. The Conformity of AIO Admixtures With USP <729> Requirements With Respect to PFAT<sub>5</sub>.

<table>
<thead>
<tr>
<th>Admixture</th>
<th>Batch C1</th>
<th></th>
<th>Batch C2</th>
<th></th>
<th>Batch S1</th>
<th></th>
<th>Batch S2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day of Preparation</td>
<td>At 24 Hours</td>
<td>Day of Preparation</td>
<td>At 24 Hours</td>
<td>Day of Preparation</td>
<td>At 24 Hours</td>
<td>Day of Preparation</td>
<td>At 24 Hours</td>
</tr>
<tr>
<td>AIO2</td>
<td>R</td>
<td>—</td>
<td>AIO2</td>
<td>A</td>
<td>—</td>
<td>—</td>
<td>AIO2</td>
<td>R</td>
</tr>
<tr>
<td>AIO3</td>
<td>R</td>
<td>—</td>
<td>AIO3</td>
<td>A</td>
<td>—</td>
<td>—</td>
<td>AIO3</td>
<td>A</td>
</tr>
<tr>
<td>AIO4</td>
<td>R</td>
<td>—</td>
<td>AIO4</td>
<td>A</td>
<td>—</td>
<td>—</td>
<td>AIO4</td>
<td>A</td>
</tr>
<tr>
<td>AIO5</td>
<td>R</td>
<td>—</td>
<td>AIO5</td>
<td>A</td>
<td>—</td>
<td>—</td>
<td>AIO5</td>
<td>A</td>
</tr>
<tr>
<td>AIO6</td>
<td>R</td>
<td>—</td>
<td>AIO6</td>
<td>A</td>
<td>—</td>
<td>—</td>
<td>AIO6</td>
<td>A</td>
</tr>
</tbody>
</table>

Bags connected to a supply pump for 24 hours at room temperature (25°C). The synthesis of admixtures AIO2-AIO6, as presented in Table 1. Batch C2 and batch S2 were free of calcium. The percentage of volume weighted particles with diameter > 5 μm. LE1 and AA1 for batch S, LE2 and AA2 for batch C. A for admixtures within acceptable limits, R for rejected admixtures, — for admixtures not assessed because they had already been rejected.

**Discussion and Conclusions**

Although there are encouraging references to AIO parenteral admixtures for neonates, a hospital pharmacy is required to assess the characteristics of every formulation prepared extemporaneously and to document if it is appropriate for clinical use.

The procedures followed in our study were identical to those proposed for clinical use. Since every parenteral formulation prepared in a hospital pharmacy has to be used within 24-30 hours to decrease the risk of contamination, and because of the unpredictable kinetic stability of AIO parenteral admixtures (associated with their synthesis), the characteristics of our admixtures were assessed on the day of preparation (within 3 hours) and then 24 hours after being connected to a supply pump, at room temperature. In addition to this, a second
A bag of each admixture was assessed after storage at 4°C for 24 hours because it is often desirable to prepare a formulation, store it, and use it later or the following day.

The addition of calcium seems to have had an undesirable affect on the admixtures in 2 ways. First, it enhanced aggregation and subsequently caused the production of sediment, and second it led to an increase of PFAT₅ (both batches S1 and C1). The latter was probably due to the aggregation of lipid droplets, a phenomenon that has been cited as the cause of emulsion destabilization. In general, this effect was expected because of the extremely high concentration of calcium and was the reason why batches S2 and C2 were also prepared.

The use of ingredients of different commercial origin seems to have produced admixtures with different characteristics. Only batch C2 admixtures (containing the lipid emulsion LE2 and the amino acid solution AA2) were within acceptable limits after 24 hours at both 4°C and 25°C in contrast to batch S2. What is more, on the day of preparation batch C2 admixtures had an MDD of 271 ± 0.8 nm compared with 295 ± 1.7 nm for batch S2 (P < .0001, unpaired t test).

Also of interest were the findings for batch C2 (Table 4); AIO1, AIO2, and AIO3 admixtures remained within the USP <729> standards, whereas AIO5 and AIO6 did not. In our opinion, vitamin additives could possibly have affected the stability of the admixtures, since only AIO5 and AIO6, the admixtures containing vitamins (water and lipid soluble), did not comply with the USP <729> standard.

In summary, all admixtures assessed were within the specifications set by the U.S. Pharmacopoeia with respect to the MDD at all time points, but only batch C2 remained within PFAT₅ limits after 24 hours; further testing is necessary to validate its appropriateness for administration. In all the other admixtures, the presence of calcium led to precipitation due to its incompatibility with the other components of the admixtures, regardless of the commercial ingredients used for their preparation. The formation of precipitates due to the presence of calcium has resulted in fatalities and should be avoided. In this context it is clear that compatibility testing of commercial products prior to the preparation of AIO admixtures is of great clinical importance.

### Table 4. The Conformity of AIO Admixtures With USP <729> Requirements With Respect to PFAT₅

<table>
<thead>
<tr>
<th>Admixture</th>
<th>Batch C1</th>
<th>Batch C2</th>
<th>Batch S1</th>
<th>Batch S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIO2</td>
<td>A R —</td>
<td>AIO2 A</td>
<td>AIO2 R</td>
<td>A R —</td>
</tr>
<tr>
<td>AIO3</td>
<td>A R —</td>
<td>AIO3 A</td>
<td>AIO3 A</td>
<td>A R —</td>
</tr>
<tr>
<td>AIO4</td>
<td>R — —</td>
<td>AIO4 A</td>
<td>AIO4 A</td>
<td>A R —</td>
</tr>
<tr>
<td>AIO5</td>
<td>R — —</td>
<td>AIO5 A</td>
<td>AIO5 A</td>
<td>A R —</td>
</tr>
<tr>
<td>AIO6</td>
<td>R — —</td>
<td>AIO6 A</td>
<td>AIO6 A</td>
<td>A R —</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Admixture</th>
<th>Day of Preparation</th>
<th>At 24 Hours</th>
<th>At 48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIO2</td>
<td>R</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AIO3</td>
<td>R</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AIO4</td>
<td>R</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AIO5</td>
<td>R</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AIO6</td>
<td>R</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Bags stored at 4°C for 24 hours, then connected to a supply pump for 24 hours, at 25°C. The synthesis of admixtures AIO2-AIO6, as presented in Table 1. Batch C2 and batch S2 were free of calcium. The percentage of volume weighted particles with diameter > 5 μm. LE1 and AA1 for batch S, LE2 and AA2 for batch C. A for admixtures within acceptable limits, R for rejected admixtures, — for admixtures not assessed because they had already been rejected.

### References


