Experience of compounding total parenteral nutrition admixtures for preterm infants in a hospital pharmacy: evidence of calcium and phosphate compatibility problem

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ABSTRACT
Objective Parenterally fed preterm newborn infants require large amounts of calcium and phosphate in a low volume of solution. The lower the volume of solution, the higher is the possibility of precipitation of calcium hydrogen phosphate (CaHPO4). Precipitation could cause respiratory distress and pulmonary embolism, and the use of organic salts of calcium and phosphorus may reduce the likelihood of this problem. To date, no previous work on the stability of solutions with organic salts has been published in the literature. This study aims to evaluate the visible precipitation of calcium and phosphorus in total parental nutrition solutions.

Methods 20 parenteral nutrition solutions were aseptically prepared in a laminar airflow hood in a clean room. The solutions are intended to facilitate precipitation, with the amino acid ratio below the standard concentration and other parameters also modulated to promote the precipitation of CaHPO4. The solutions contained dextrose, amino acids, calcium gluconate and fructose 1,6-bisphosphate. We did not use lipid emulsion so that we could see all precipitations.

Results No visible precipitation was observed during 4 weeks of observation at 25°C. The only observed event was the change in colour of the solution, which became yellow, maybe because of a Maillard reaction.

Conclusions This study evaluated the compatibility of organic calcium and phosphorus in order to prevent the precipitation of CaHPO4 when preparing total parenteral nutrition solutions. The fact that no precipitation was observed is very significant as it indicates the compatibility of the ions, even though no instrumental analysis was performed.

INTRODUCTION
The American Academy of Pediatrics recommends that, after birth, preterm infants should grow as a normal fetus of the same gestational age.1 The minimum rate of growth is 15 g/kg/day during mid-trimester and 10 g/kg/day at term.2 Often this weight gain is not achievable in preterm infants because extrauterine life requires higher energy expenditure than intrauterine life.2 Breast milk feeding remains the best way to provide nutrition to the newborn infant, but it is not always possible to start breast feeding immediately after the delivery of a preterm infant3 because coordination of sucking/swallowing and swallowing/breathing does not occur before 32 and 33–34 gestational weeks, respectively.3

Therefore, in order to achieve a neonatal growth rate similar to a normal fetus, early parenteral nutrition (PN) should be started immediately after birth.2 PN is often used in association with minimal enteral feeding; this permits the administration of low volumes of enteral nutrition to the infant. Enteral nutrition stimulates the development of the gastrointestinal tract, thus improving enzymatic activity, hormonal release, blood flow, intestinal motility and flora.4

The Italian Society of Paediatrics5 has established that the initial energy requirement of preterm infants is 45–65 kcal/kg/day with a protein:energy ratio of 1:2.5. Table 1 (modified from table 1 published by the Italian Society of Paediatrics6) shows the initial and final requirements of preterm infants. As can be seen from the table, large amounts of calcium (Ca2+) and phosphate (PO4)3– are needed as these two ions are necessary for the mineralisation of bone. The optimal Ca2+:P ratio is 1.7:1.6 which is the same as the ratio found in human breast milk.

The problem is intensified by the fact that preterm infants often suffer from oliguria. In such cases, the volume of PN administered should be as low as possible, but the lower the volume, the higher is the risk of precipitation of calcium hydrogen phosphate (CaHPO4).

In 1994, after two cases of death due to pulmonary embolism and two cases of pulmonary distress developed during peripheral infusion of all-in-one total parenteral nutrition (TPN) admixtures, the Food and Drug Administration (FDA) published a public safety alert.7 According to the FDA, the following parameters should be verified prior to administering TPN:

- pH
- Ca2+ and (PO4)3– concentration and their solubility, which should be calculated from the volume at the time the Ca2+ is added
- Phosphate content of amino acid solution
- Order of mixing
- Filtration when infusing
- Visual inspection during the mixing process and before infusing
- Time of administration: if stored at room temperature the infusion should start within 24 hours after mixing; if stored at refrigerated temperatures the infusion should start within 24 hours after being warmed.
pH plays a very important role. The solubility of calcium dihydrogen phosphate (Ca(H$_2$PO$_4$)$_2$) is 1.8 g/L while the solubility of CaHPO$_4$ is 0.03 g/L. According to the Henderson–Hasselback equation, at pH 7.4, 60% of phosphate is in the bissac form, (HPO$_4$)$_2^{-}$, which is less soluble. By reducing the pH by 2 units, 95% of phosphate is in the monobasic form, (H$_2$PO$_4$)$^-$, which is far more soluble.8

The concentration of amino acids is the factor that most influences the pH of the final admixture. Amino acids are able to buffer the solution, depending on the concentration of arginine, histidine and lysine.9 Raising the final concentration of amino acid in the admixture increases the buffering capacity of amino acids.10–12 The minimum concentration usually used in paediatric PN is 2.5–3.5%. This value represents the required concentration to exert an effective buffering effect in the TPN admixture.

Lenz and Milkrut12 underlined the importance of cysteine in reducing the pH of the admixture, with a stabilising effect. In a solution without cysteine, with 2.5% of amino acids and 10 mmol/L calcium chloride (CaCl$_2$), the maximum amount of inorganic phosphate as Na$_2$PO$_4$ is 7.5 mmol/L. In admixtures with cysteine (50 mg/dL), the compatible quantity of Ca$^{2+}$ and phosphate rises to 10 mmol/L, thus reducing the medium Z potential of the particles.13

In addition to the buffering effect, amino acids can chelate Ca$^{2+}$. In this way, amino acids can reduce the free rate of the cation.14 Amino acids such as glutamic acid, aspartic acid, arginine, histidine and lysine are able to bind the ion Ca$^{2+}$.15

Even the amount of dextrose affects the pH of the final solution, but to a less significant extent.16

The FDA recommends that an admixture should be administered within 24 hours after warming.7 Controlling and storing the admixture at a regulated temperature helps to prevent the precipitation of CaHPO$_4$. In fact, the temperature is able to influence the dissociation of the calcium salt used, often Ca$^{2+}$ gluconate, thus increasing the free rate of Ca$^{2+}$ in the admixture.17

Increasing the temperature enhances the quantity of the phosphate ion in the monobasic form. Thus, the concentration of (HPO$_4$)$_2^{-}$ increases, being less soluble, while the concentration of the monobasic form (H$_2$PO$_4$)$^-$ decreases, being more soluble.15

Also, the order of adding Ca$^{2+}$ and phosphorus (P) is very important and must follow a particular order. P must be added to the bag and vigorously mixed before adding Ca$^{2+}$.9 Di Salvo14 suggested adding Ca$^{2+}$ and P to different solutions: Ca$^{2+}$ should be added to the solution of amino acids in order to decrease the free rate of the ion, while P should be added to the hypertonic dextrose solution that promotes the formation of monobasic phosphate, which is more soluble than the bissac form. However, the infusion of two bags will increase the number of manipulations required when administering the solution, increasing the risk of contamination.18

The choice of salt used plays an important role in the stability of TPN admixtures. CaCl$_2$ is the inorganic source of Ca$^{2+}$. Given that CaCl$_2$ is more dissociated than the organic salt (Ca$^{2+}$ gluconate), CaCl$_2$ reduces the compatibility of Ca$^{2+}$ and P because of a major concentration of free Ca$^{2+}$.19 Because of this, it is suggested that an organic Ca$^{2+}$ salt such as Ca$^{2+}$ gluconate should be used. The anion gluconate has a larger steric effect than Cl$^-$, reducing the dissociation constant of the salt. This effect limits the quantity of free Ca$^{2+}$ in the admixture.

With regard to phosphate salts, there are commercially available organic sources of P such as glucose-1-phosphate, glycerol-3-phosphate and fructose-1,6-bisphosphate (FDP). All have a phosphoester bond, which makes the P organic.

FDP is a physiological metabolite of glycolysis20 and can also be used in cases of hypophosphataemia during transfusion therapies, extracorporeal circulation, chronic alcoholism, lung failure and prolonged malnutrition. This phosphate group donor is easier to handle than inorganic ions because it has better compatibility with Ca$^{2+}$.21

In addition, FDP has the following benefits in vivo:15

- It skips three steps of glycolysis, saving two molecules of ATP.
- It keeps a high level of 2,3-bisphosphoglyceric acid in the red blood cells, which enhances the release of oxygen to the peripheral tissues.
- It is slowly eliminated from the kidneys.

The last parameter analysed is the final concentration of bivalent ions such as Mg$^{2+}$, Cu$^{2+}$, Zn$^{2+}$ and Mn$^{2+}$ in the admixture. Schuetz and Kins12 suggest that the Mg$^{2+}$ ion is able to influence the likelihood of precipitation because it forms relatively more soluble and stable salts with phosphate ions. Jimenez-Torres and Ronchera-Oms16 indicate that ratios of Ca$^{2+}$:Mg$^{2+} < 2$ exert a positive effect on CaHPO$_4$ solubility.

Our analysis focuses on the compatibility of organic Ca$^{2+}$ as gluconate and P as FDP, since so far we have not found any studies evaluating the compatibility of these ions in organic form in TPN admixtures. The scope was to evaluate the concentration of organic Ca$^{2+}$ and organic P that would show evidence of a visible precipitation in the solution. We produced admixtures with a fixed concentration of amino acids of 2%, not beyond the fixed minimum standard concentration of 2.5%. In a second experiment, we changed factors such as dextrose, organic–inorganic salt and the concentration of bivalent ions in order to determine which factors are capable of facilitating the precipitation of CaHPO$_4$.

**METHODS**

TPN admixtures were prepared aseptically following international recommendations under a laminar airflow hood in a clean room at the Total Parenteral Nutrition Laboratory of ASST Bergamo Est Hospital. Ethyl vinyl acetate (EVA) bags (Aries) were manually prepared in order to analyse the compatibility of Ca$^{2+}$ and P. No lipids were added because they would obscure the presence of a precipitate.
We prepared the first four bags using TPH 6% (Baxter) as this product represents the pattern of amino acids in the preterm baby. Because of a low concentration of TPH 6%, we could not standardise the solution to 100 mL with high volumes of Ca\(^{2+}\) and P solutions. After a comparison of the composition of TPH 6% and Sintamin 10% (Fresenius Kabi), we decided to change to Sintamin 10% because of its higher concentration of amino acids. As shown in table 2, Sintamin 10% allowed us to reach the standard 2% of amino acids while keeping the standard volume of 100 mL. Despite the fact that TPH 6% contains amino acids such as aspartate, glutamic acid, tyrosine and taurine, the composition of Sintamin is quite similar. Indeed, as shown by Driscoll et al., the content of Ca\(^{2+}\)-binding amino acids (arginine, histidine and lysine) is equivalent. Even the cysteine composition is similar between TPH 6% and Sintamin 10%; TPH contains 0.33% of the sulfur amino acid while Sintamin contains 0.32%. As stated earlier, cysteine is able to influence the pH and, consequently, the compatibility of Ca\(^{2+}\) and phosphate.\(^{14}\)

After this evaluation we decided to use Sintamin 10% in a final concentration of 2%, which is lower than the standard range of amino acids (2.5–3.5%), in order to promote the precipitation of CaHPO\(_4\).

We analysed the compatibility of the organic salts of Ca\(^{2+}\) and P using Ca\(^{2+}\) gluconate 10% (0.446 mEq/mL; Monico) and FDP 10% (0.47 mEq/mL of P; Esafosha, Biomedica Foscoma). However, bags P15, P16 and P17 contain CaCl\(_2\) 10% (1.36 mEq/mL; Monico) because we wanted to reach a higher Ca\(^{2+}\) concentration with a lower Ca\(^{2+}\) solution volume in the same final 100 mL.

Our TPN solutions were prepared with the aim of containing 2% of amino acids, 10% of dextrose (from dextrose 50%; Baxter). We then tried to add Mg\(^{2+}\) (Mg sulfate 0.5 mmol/mL; Monico). To analyse the compatibility with more bivalent ions, we also added Zn\(^{2+}\) to the admixtures, alone or together with Mg\(^{2+}\).

### Table 2 Comparison between Sintamin 10% and TPH 6%

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Sintamin 10% (%)</th>
<th>TPH 6% (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>6.68</td>
<td>5.32</td>
</tr>
<tr>
<td>Arginine</td>
<td>9.02</td>
<td>12.14</td>
</tr>
<tr>
<td>Asparagine</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td>Glycine</td>
<td>10.96</td>
<td>3.66</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4.99</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>6.24</td>
<td>7.81</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.85</td>
<td>3.33</td>
</tr>
<tr>
<td>Phenyalaniline</td>
<td>5.32</td>
<td>4.82</td>
</tr>
<tr>
<td>Proline</td>
<td>10.58</td>
<td>6.82</td>
</tr>
<tr>
<td>Serine</td>
<td>5.77</td>
<td>3.82</td>
</tr>
</tbody>
</table>

The details of the first four bags (P1–P4) are shown in table 3. TPH 6% was used but, as can be seen from the table, the volume was too high. P1 does not contain dextrose in order to evaluate how sugar affects the stability and compatibility.

Table 4 shows the details of the next five bags (P5–P9). From P5 we started to use Sintamin 10%. The concentration of dextrose was 10% in all the bags, except for P8 which was prepared without dextrose. In order to analyse the effect of amino acids, all bags contained 2% of amino acids, except for P9 which was produced without Sintamin 10%. The variations between P8 and P9 are to evaluate how dextrose and amino acids can influence the precipitation of CaHPO\(_4\). Details of the last 11 bags (P10–P20) are shown in table 5. These admixtures contain Mg\(^{2+}\) in a Ca\(^{2+}\):Mg\(^{2+}\) rate of >2. This rate reduces the compatible concentrations of Ca\(^{2+}\) and P, as shown by Boulet and Marier.\(^{21}\)

Also, a bag without dextrose (P14) and a bag without amino acids (P13) were prepared to evaluate the effect of dextrose and amino acids. To reach a higher concentration of Ca\(^{2+}\) in the bag while maintaining the final volume at 100 mL, we made three bags with CaCl\(_2\) (P15, P16 and P17), as shown in table 5. As this is an inorganic salt, we thought that its higher dissociation rate would facilitate the precipitation of CaHPO\(_4\), thus enabling us to compare our work. The last three bags (P18–P20) also contained Zn\(^{2+}\) in order to increase the quantity of bivalent ions and analyse their effect on the stability of the admixtures (table 5).

### RESULTS

We evaluated immediate and late precipitation. No visible precipitation occurred in either the late or immediate evaluation. A limitation of our study is the absence of instrumental analysis.
DISCUSSION

The aim of this study was to evaluate the compatible amount of organic Ca\(^{2+}\) and P in TPN admixtures because, to date, there has been no study of the use of these two organic salts in the literature. High Ca\(^{2+}\) and high P admixtures were produced to check whether precipitation occurred. Even though we changed many parameters that might make the admixture unstable, CaHPO\(_4\) precipitation was not observed.

This study suggests that the use of organic sources of Ca\(^{2+}\) and P enhances the compatibility of the two ions. In order to confirm this hypothesis more evaluations are needed, such as a potential Z analysis or a particle count. It would be interesting to analyze a solution of the same composition using a particle counting machine and HPLC. The next step in our analysis is to evaluate the compatibility of Ca\(^{2+}\) and lipid emulsions.

What this paper adds

This study evaluated the compatibility (and therefore the stability) of total parenteral admixtures containing organic calcium (calcium gluconate) and organic phosphorus (fructose 1,6-bisphosphate). The aim of the study is to evaluate the stability of admixtures containing high concentrations of calcium and phosphorus and to check how some parameters influence the stability of total parenteral nutrition bags.

REFERENCES


