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Research Paper

Effect of the Intravenous Lipid Emulsions on the Availability of Calcium when using Organic Phosphate in TPN Admixtures

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Purpose. The addition of high amounts of calcium remains a pharmaceutical concern due to its precipitation with phosphate in total parenteral nutrient (TPN) admixtures, compromising also the stability of the lipid emulsion.
Materials and Methods. Calcium-phosphate solubility was compared when using binary PN solutions *versus* all-in-one TPN (admixtures with lipid emulsions) in three formulas using organic calcium gluconate and gulcose-1-phosphate.
Results. It was found that variation of Ca–P solubility exists between formulation with or without lipid emulsions. Concentrations of Ca decreased after filtrations of all admixtures (from 5% to 30%) and it was more significant in binary solutions. Precipitation has been observed by microscopy at high concentrations of both organic Ca–P after critical conditions of storage (24 h at 37°C plus one day at ambient temperature) for admixtures containing 1% amino acids and 8% glucose with or without lipids compared to admixtures containing 2% or 3.5% amino acids and 14% glucose.
Conclusions. These data demonstrated that availability of Ca using organic glucose-1-phosphate increased when lipids were present in TPN admixtures, without alteration of the lipid emulsion. Thus, high amounts of Ca (up to 30 mmol/l) and phosphates (up to 40 mmol/l) might be provided safely in parenteral nutrition admixtures.

KEY WORDS: calcium; compatibility; parenteral nutrition; phosphate; stability.

INTRODUCTION

The addition of high concentrations of calcium (Ca) and phosphate (P) to insure an optimal delivery of calcium for a restricted fluid volume remains an important pharmaceutical concern in the manufacturing of total parenteral nutrition (TPN) admixtures (1–4).

Clinical complications and deaths due to calcium gluconate and potassium phosphate interaction have been reported (5), other reports published in 1996 (6) indicated suspect death resulted from an incompatibility between calcium and phosphate salts associated with the mixing of total parenteral nutrition formulations. Also, the US Food and Drug Administration (FDA) published a Safety Alert report in 1994 (7).

Numerous studies have attempted to determine the maximum concentrations of various combinations of calcium and phosphorus that can be mixed safely, but the results obtained cannot be universally applied because of the differences in the study conditions and the variety of compounding products used (8–11). Crystallisation of Ca–P is more likely to occur at lower volumes when the solution is warmed to body temperature in the central venous catheter (12) or in incubators used for neonates because calcium-phosphate reaction is endothermic (13). In fact, the increase of temperature will have two effects, one on the dissociation of calcium from its organic form; this will increase the availability of free Ca which will react with phosphate. Second, raising the temperature of a mixture may also shift the phosphate equilibrium from mono- to dibasic salt. Both effects increase the likelihood of precipitation (4). Visual inspection can sometimes be ineffective in preventing infusion of the precipitate, especially for all-in-one admixtures in which lipid emulsions are presented (4).

Several factors can affect the solubility of these two electrolytes (1–4) including the concentration of calcium and phosphate ions, salt of phosphate or calcium used, composition and concentration of amino acid solutions, temperature

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ABBREVIATIONS: CaGlu, calcium gluconate; G1P, glucose-1-phosphate; IV, intravenous; IVLEs, intravenous lipid emulsions; PN, parenteral nutrition; TPN, total parenteral nutrition.

Table I. Composition of Standard Solutions N1, N2 and N3

Additive (concentration)	Formula N1	N2	N3
Glucose (g/100 ml)	8	8	14
Amino acids (g/100 ml)[a]	1	2	3.5
Sodium (mmol/100 ml)[b]	3	3	3
Potassium (mmol/100 ml)[c]	2	2	2
Magnesium (mmol/100 ml)[d]	0.4	0.4	0.4
Water for injection	qsp	100 ml	

[a] Primene® 10% solution (composition: AA: 10 g/100 ml; Cl^-: 15.6 mmol/L; Na^+ 5 mmol/L; Baxter, France)
[b] NaCl 10% injectable solution (1 ml=1.7 mmol NaCl; Unimed, Tunisia)
[c] KCl 7.46% injectable solution (1 ml=1 mmol KCl; Siphat, Tunisia)
[d] Magnesium sulfate 15% injectable solution (1 ml=0.61 mmol Mg; Aguettant, France)

and pH of solutions, presence of other electrolytes and additives, order of mixing, infusion rates and length of storage. The dibasic calcium phosphate salt is 60 times less soluble than the monobasic form in aqueous medium (3) and its solubility is highly pH-dependant (4). Furthermore, the use of organic phosphates in the form of sodium glucose-1-phosphate (14), sodium glycerophosphate (9) or sodium d-fructose1,6-diphosphate (15,16) considerably increases the Ca–P compatibility. These studies confirmed that the organic phosphate has superior stability in comparison to dibasic sodium phosphate over a range of pH, temperature, calcium used and aminoacid or glucose concentrations.

A systematic comparative *in vitro* assessment of the therapeutic potential and safety of organic phosphates in the parenteral nutrition of premature infants (9) suggested that glycerophosphate, and particularly glucose-1-phosphate, together with calcium gluconate, is an uncomplicated and safe way to administer simultaneously high amounts of calcium and phosphorus in TPN solutions. Furthermore, organic calcium additives (Ca-gluconate or glucoheptonate) have lower degree of dissociation compared to inorganic calcium chloride (17). Mixtures containing 33.3 mmol/l of $CaCl_2$ and 10 mmol/l of d-fructose 1,6-diphosphate were stable at 37°C and 2% amino acid (18).

It was suggested that the probability of calcium and phosphate (Ca–P) precipitation is heightened when additives are incorporated into all-in-one lipid containing admixtures intended for very young (18). Whether or not lipid contributes to Ca–P stability or vice versa is complex and still debatable. One argument suggests that Ca binding to the phospholipid emulsifier limits the chance of Ca and P interaction thus reducing the likelihood of precipitation.

All-in-one admixtures are complex pharmaceutical preparations containing fat emulsions. Commercially available intravenous lipid emulsions (IVLEs) are two phase system, generally viewed as a dispersion of sub micron oil droplets surrounded by single or monomolecular layer of surfactant. These are stabilized by a mixture of phosphatides derived from egg lecithin that collectively produces a highly effective emulsifier with a net anionic charge at the surface of the lipid droplets (19,20).

Several factors are responsible of the degradation of the lipid emulsions. First, the addition of cationic electrolytes, particularly those of higher valence such as calcium and magnesium, will interact with the anionic emulsifying agent present at the surface of the lipid globules (19–21). Second, the reduction of the inter-globular distance will increase the attractive forces between oil droplets, resulting in the immobilisation of the globules at a minimum distance to the repulsive forces. Both ways of degradation described will result in droplets aggregation (or flocculation which is a reversible stage), then globule coalescence and finally phase separation.

Considering the potential risk to have precipitations and problems associated to the stability of the lipid emulsions related to the use of high concentrations of calcium in neonatal parenteral nutrition solutions or in TPN admixtures. This study compare the effect of the addition of the lipid emulsions on the Ca and P solubility when using organic phosphate (glucose-1-phosphate) and Primene® as amino acid source under simulated clinical conditions.

MATERIAL AND METHODS

The study concerned three standard neonatal PN admixtures named N1, N2 and N3 currently used (except for the formula N1) at the Maternity Hospital. The compositions of these formulas are presented in Table I. Duplicate samples of each solution were prepared in a 1000 ml ethylene vinyl acetate (Nutripoche® EVA M) parenteral nutrition (PN) bag (Baxter, France) using aseptic techniques in a vertical laminar air flow hood. Preliminary studies showed no degradation of the physicochemical stability of these standard solutions after storage at −40°C. Samples were removed from the freezer and stored at room temperature 4 h prior to commencing the experiments. The organic source of phosphorus used is Phocytan® (glucose-1-phosphate) supplied by Aguettant, France. One millilitre of this solution contains 0.33 mmol of phosphate. Calcium gluconate at 10% (Glucalcium® Renaudin, France) was used as an organic calcium source. ClinOleic® and Ivelip®: were used as lipid emulsion source and both are supplied by Baxter Clintec Parenteral SA (France). The first one is based upon a mixture of olive oil which is rich in the n-9 monounsaturated fatty acid and soybean oil (80:20), the second is essentially composed of soybean 20% which is rich in the n-6 polyunsaturated fatty acid (PUFA) linoleic acid (18:2n-6), complete compositions are indicated in Table II.

Table II. Composition and Characteristics of the Commercial Clinoleic® 20% Emulsion Compared to Ivelip® 20% Emulsion

	Clinoleic®	Ivelip®
Purified olive oil	80%	0%
Soybean	20%	100%
Essential fatty acids	200 g/l	200 g/l
Purified egg yolk phosphatides	12 g/l	12 g/l
Sodium oleate	0.3 g/l	0.3 g/l
Glycerol	22.5 g/l	25.0 g/l
Sodium hydroxide	Qs pH 7–9	qs pH 6–8
Osmolarity	270 mOsm/l	270 mOsm/l
Mean particle size	0.3–0.4 μm	0.25–0.4 μm
Energy content	2,000 kcal/l (8,360 kj)	2,000 kcal/l (8,360 kj)

General Procedure

Two series of experiments were prepared. First series (N1, N2 and N3 samples): were PN admixtures composed of binary solutions without lipids. Second series (TPN admixtures): were the same admixtures to which lipid emulsions were added to each sample to obtain a final concentration of 2 g of fat per 100 ml of the solution.

For each series, appropriate volumes of the standard solution were withdrawn and transferred to universal 20 ml glass test tube using 0.22 μm filter (Sterifix® Luer Lock, Braun, France; $n=29$ for the PN series and $n=58$ for TPN series).

Then to provide cationic stress out of the clinical limits, the two electrolytes calcium and phosphate, were given in amounts equal from two to three times their usual dose. For that, three consecutives concentrations of the glucose-1-phosphate (G1P) solutions (26, 32 or 40 mmol/l of phosphate) were added to the samples. The tubes were shaken and calcium (Ca) at concentrations of 15.5, 24.5 and 30 mmol/l as calcium gluconate (CaGlu), were added respectively to each concentration of G1P, as presented in Table III. The samples were then capped and vigorously shaken to mix and eliminate local concentrations of both calcium and phosphate.

Appropriate volume of lipid (alternatively ClinOleic® or Ivelip®) was added to the second series of experiments to obtain 2% (*w*/*v*) of fat in each sample. The phosphorus content of the original lipid emulsions (as indicated in the monography of Clinoleic®) is equal to 1.5 mmol/100 ml or 47 mg/100 ml. This represents 0.03 mmol/100 ml (=0.94 mg/100 ml) of the final admixtures in the ternary solutions studied. This amount is very low compared to the concentration of phosphate added to our samples as Phocytan® (glucose-1-phosphate) and was not been taken into account in the calculation of P content of the ternary samples.

Two Blank samples for each series were prepared; Blank 1 containing all ingredients without phosphates and Blank 2 containing all ingredients without neither calcium nor phosphate. All samples were allowed to stand undisturbed at 37± 1°C for 24 h.

Parameters Controlled

Visual Observation

Immediately after the addition of P and Ca (at T0) and at the end of the storage period after 24 h at 37±1°C (T1), the PN samples were visually inspected using good illumination conditions (light of high intensity) against dark back-ground for evidence of precipitation or crystallization. Also, detailed visual analysis was performed to TPN samples for evidence of heavy creaming and phase separation with the liberation of free oil.

Microscopic Examination

Binary PN solution samples were then checked microscopically for evidence of micro crystallisation, using a Zeiss Axioskop microscope (Germany) connected to a digital camera (Canon, France) with polarizer at power 400×, and TPN samples were examined at magnification 100×/1.25 oil immersions, fitted with a graticule micrometer.

pH Measurement

The pH of each sample was measured with Hanna® (USA) instruments pH-meter after the time storage. The pH-meter was calibrated before each series of measurement at pH 4 and 7.

Calcium Determination

Ca concentrations were determined by atomic absorption spectroscopy (Perkin-Elmer 305 B, USA) before and after filtration (using 0.22 μm for the PN samples and 0.45 μm for TPN samples). The linearity of the method was performed from 1 to 10 mg/l by using a 0.1% Lanthanum oxide solution, in order to eliminate interferences with other metals during the dosage of calcium. The samples were diluted to 1/200 and 1/300 before analysis.

Particle Size Analysis

Particle size was assessed on a Nanosizer® ZS (Malvern Instruments, UK) at 25°C without dilution to detect particles from 0.6 nm to 6 μm, and by a Mastersizer® (Malvern Instrument, UK). In this case the sample was diluted automatically in deionised water for the detection of particles greater than 6 μm.

Zeta Potential Measurement

To verify the potential stability of the colloidal system, zeta potential was determined for the TPN samples with the same Nanosizer® ZS instrument, by measuring the drift velocity of the particle in an electrical field of known strength.

Table III. Composition of the Samples to be Tested after the Addition of Ca–P and Lipids (Final Volume 20 ml)

Samples	1	2	3	4	5	6	7	8	9	Blank 1	Blank 2
PN solutions											
Solution to be tested N1, N2 or N3 in ml	12.8	15.3	16.2	15.3	15.8	16.6	15.7	16.2	17.0	17.2	20
P as Phocytan (in mmol/l)	40	40	40	32	32	32	26	26	26	0	0
Ca as CaGluconate (in mmol/l)	30	24.5	15.5	30	24.5	15.5	30	24.5	15.5	30	0
Lipids (g/100 ml) (Clinoleic or Ivelip)	–	–	–	–	–	–	–	–	–	–	–
TPN admixtures											
Solution to be tested N1, N2 or N3 in ml	10.8	13.3	14.2	13.3	13.8	14.6	13.7	14.2	15.0	15.2	18
P as Phocytan (in mmol/l)	40	40	40	32	32	32	26	26	26	0	0
Ca as CaGluconate (in mmol/l)	30	24.5	15.5	30	24.5	15.5	30	24.5	15.5	30	0
Lipids (g/100 ml) (Clinoleic or Ivelip)	2	2	2	2	2	2	2	2	2	2	2

Considering water as the disperse phase of the original emulsion samples, samples were diluted to 1% (*v*/*v*) in deionised water prior to be tested. The magnitude of the zeta potential gives an indication of the stability of the emulsion.

Statistical Analysis

Statistical analysis was performed by using non parametric ANOVA (Kruskal–Wallis test) for comparison of multiple means related to pH and Ca determination before and after filtration, when comparing binary solutions *versus* ternary TPN admixtures. Differences were considered as significant when $p<0.05$. We used non parametric test because sample sizes were reduced and the data were not normally distributed. For each determination, samples were tested in triplicate. We used non parametric test because sample sizes were reduced ($n=29$ for the PN series and $n=58$ for TPN series) and the data were not normally distributed.

RESULTS

Visual Appearance

The results revealed that the binary (PN) solutions remained clear after 24 h of storage at 37±1°C (at T1), even at high calcium and phosphates concentrations (Ca up to 40 mmol/l and P up to 30 mmol/l). Also, visual examination of the TPN admixtures revealed that all samples studied remained homogenous immediately after preparation (at T0) and after the periods of storage (at T1).

Microscopic Assessment

The microscopic investigations of the samples containing Ca and P at T1 did not reveal any difference when compared to samples at T0 for PN samples. TPN admixtures have homogenous distribution of globules (<1 μm) at T0 and in some samples made of N1 formula, greater sizes were detected ranging from 2 to 5 μm at T1 without exceeding 5 μm. Furthermore, after one more day of storage at ambient temperature, the presence of crystals with different shapes and sizes for higher Ca and P concentrations has been observed in N1 formulas for both series i.e. binary PN solutions and ternary TPN admixtures (Fig. 1A,B). Their sizes varied from 2 to 60 μm in PN solutions and between 5 and 6 μm for TPN samples.

pH Study

The pH values of the standard solutions (without calcium and phosphate) were maintained without significant changes (pH=5.01±0.05) despite the variation in their composition, and the presence of lipid emulsions did not modify the pH of these solutions.

When adding calcium and phosphate, the pH of the PN admixtures rose to 6.75±0.16, 6.55±0.12 and 6.15±0.16 (respectively for N1, N2 and N3 formulas) solutions (Fig. 2). This raising in the pH was probably due to the buffer effect of the glucose-1-phosphate. The pH of the glucose-1-phosphate product ranged between 8.5 and 8.6. The means pH corresponding to N1, N2 and N3 with various Ca–P concentrations, were found significantly different ($p<10^{-3}$) essentially due to the effect of Primene® and Glucose concentrations.

The pH raised to 6.93±0.12, 6.69±0.11 and 6.22±0.08 respectively for N1, N2 and N3 formulae with Clinoleic® and to 6.65±0.07, 6.48±0.16 and 6.18±0.08 respectively for N1, N2 and N3 formulae with Ivelip® as presented in Fig. 2. Comparison of the means pH corresponding to N1, N2 and N3 formulae with Clinoleic® or Ivelip®, were found significantly different ($p<10^{-3}$) for N1 and N2 formulas. Furthermore, the pH of the N3 admixtures was significantly decreased compared to N1 and N2 admixtures, indicating that the buffering effect of phosphates is limited at high Primene® and glucose concentrations. However, in this case the pH remained within the recommended value (pH between 6 and 7) for all samples tested (18). In fact, the pH of Primene® is 5.5 because of its amino acid composition which are more stable at this pH.

Calcium Determination

The results illustrated in Tables IV, V and VI showed a significant ($p=0.005$) decrease of Ca concentration after filtration in all solutions stored 24 h at 37±1°C, when G1P is present in the mixture, compared to the blank samples without P. Our results showed also a more significant decrease of Ca in binary solutions before filtration compared to the same admixtures containing lipids (as indicated in Tables IV, V and VI).

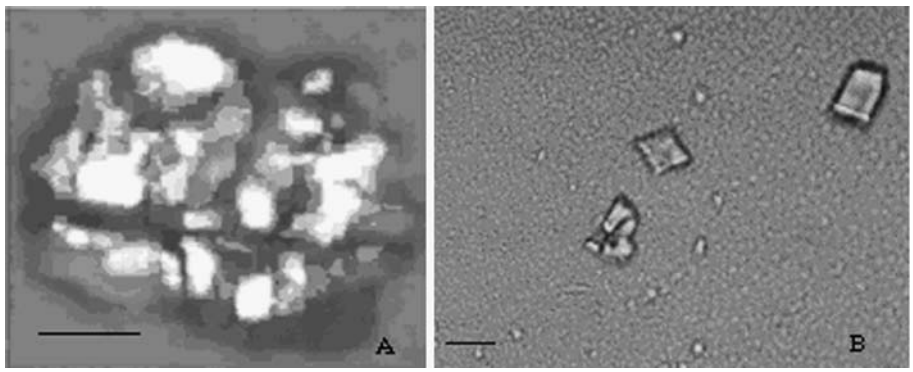

Fig. 1. Microscopic examination of the admixtures containing calcium (30 mmol/l) and phosphate (40 mmol/l) after 24 h of storage at 37°C and one day at ambient temperature. **A** In PN solutions and **B** in TPN admixtures (bar represents 5 μm).

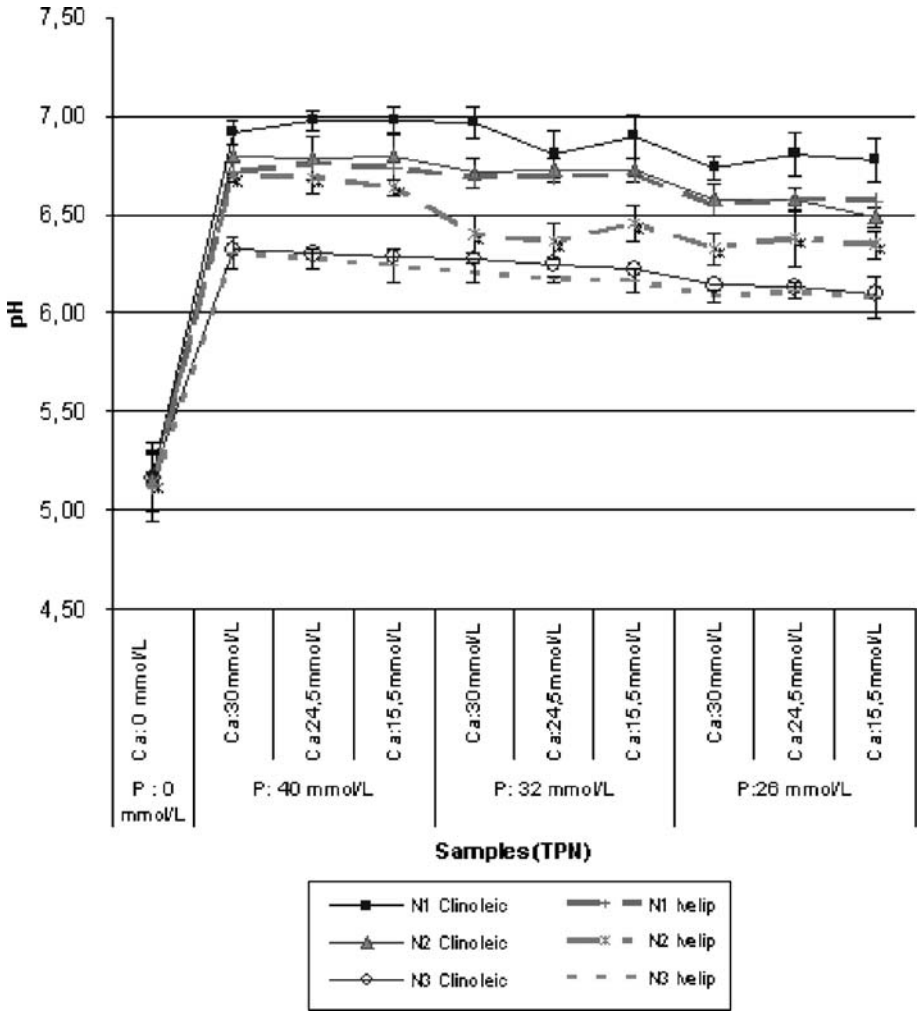

Fig. 2. Mean pH (±SD) of the TPN admixtures corresponding to N1, N2 and N3 with Clinoleic® and Ivelip® emulsions and various concentrations of Phocytan® and Ca gluconate.

In the PN solutions, the calcium concentration decreased about 20.5%, 17.5% and 16.8% after filtration for respectively N1, N2 and N3 admixtures with various Ca–P concentrations, comparing to the same solutions before filtration. The decrease in Ca concentration was 10% before filtration and 29% after filtration for N1 solutions, 5% before filtration and 12% after filtration for both N2 and N3 admixtures with various Ca–P concentrations, compared to the blank samples

Table IV. Calcium Concentrations (Expressed in mmol/l±SD) before and after Filtration (0.22 μm) of N1, N2 and N3 Solutions after 24 h of Storage at 37±1°C (n=3)

	P	Ca	Calcium concentrations (mmol/l)					
Samples	Phocytan	Ca Gluconate	Before filtration			After filtration		
N	mmol/l	mmol/l	N1	N2	N3	N1	N2	N3
1	40	30	27.10±2.02	28.48±2.40	28.95±2.91	20.72±3.54	22.46±2.23	23.97±3.25
2	40	24.5	21.38±2.22	22.44±1.41	22.95±1.47	16.90±2.88	18.34±3.21	18.62±1.97
3	40	15.5	13.79±2.67	14.51±1.56	14.56±1.97	10.59±1.61	11.37±258	12.31±1.39
4	32	30	27.55±2.11	28.15±2.52	28.59±3.21	22.49±2.60	23.99±2.23	23.62±3.30
5	32	24.5	21.69±2.04	22.77±0.75	23.00±1.54	16.89±2.01	18.54±1.19	19.63±2.61
6	32	15.5	13.43±2.55	15.06±1.50	15.51±1.91	10.61±2.10	11.64±2.21	13.08±3.30
7	26	30	28.63±1.99	28.62±2.13	29.27±2.22	23.58±2.03	24.44±1.45	25.75±2.64
8	26	24.5	22.44±2.70	23.26±1.09	23.21±1.14	17.39±2.75	19.00±2.19	19.65±2.46
9	26	15.5	14.70±1.36	14.79±1.80	14.82±1.13	11.62±1.04	11.34±1.72	12.45±1.42
Blank 1	0	30	29.43±2.15	29.38±1.38	30.07±1.53	28.59±1.01	28.22±1.82	28.20±1.93
Blank 2	0	0	0	0	0	0	0	0

Table V. Calcium Concentrations (Expressed in mmol/l±SD) Before and After Filtration (0.45 μm) of TPN (N1, N2 and N3 with 2% Clinoleic®) after 24 H of Storage at 37±1°C (n=3)

Samples	P	Ca	Calcium concentrations (mmol/l)					
	Phocytan®	Ca Gluconate	Before filtration			After filtration		
N	mmol/l	mmol/l	N1 Clinoleic®	N2 Clinoleic®	N3 Clinoleic®	N1 Clinoleic®	N2 Clinoleic®	N3 Clinoleic®
1	40	30	29.51±2.02	30.02±1.43	30.56±−1.35	23.23±3.01	25.12±2.13	24.69±2.51
2	40	24.5	24.39±1.94	24.37±1.27	24.20±2.05	19.09±2.95	18.89±1.21	19.50±1.13
3	40	15.5	14.85±1.55	15.90±1.67	15.74±1.20	12.26±0.77	10.95±0.30	12.98±2.01
4	32	30	29.65±1.46	30.82±1.55	30.03±1.92	23.58±3.26	24.62±2.44	25.45±2.36
5	32	24.5	24.19±1.32	24.25±1.09	24.27±1.87	18.86±1.35	19.32±1.82	20.70±2.61
6	32	15.5	14.74±1.20	15.20±0.73	15.63±1.67	13.27±1.86	11.88±1.21	13.27±1.86
7	26	30	29.90±1.90	30.95±1..32	30.08±1.45	23.85±1.95	24.48±2.54	25.45±4.28
8	26	24.5	24.28±1.22	24.35±1.09	24.40±1.76	19.65±2.22	19.75±2.43	20.48±1.73
9	26	15.5	15.32±0.84	15.49±1.33	15.18±1.67	12.54±0.86	12.25±1.82	13.56±1.82
Blank 1	0	30	29.70±1.77	29.82±1.35	29.33±1.23	27.15±1.53	27.91±1.64	27.36±1.53
Blank 2	0	0	0	0	0	0	0	0

without phosphate. While Ca concentrations were maintained stable in all TPN emulsion samples at T1, no decrease were observed before filtration (Tables V and VI). In this case, the decrease of Ca after filtration represented the same proportions as for PN solutions. The decrease of calcium after filtration represented less than 5%, in all mixtures without phosphate and was more important in TPN samples compared to PN solutions. Moreover, during the filtration process, the filters were gradually but not entirely blocked and all solutions were completely filtered.

These results indicated that Ca–P precipitation occurred in all formulas tested but it was more pronounced for samples containing the highest concentrations of both ions, for PN solutions before filtration and for the N1 admixtures containing the lowest concentration of amino acids and higher pH level.

Particle Size Determination

With regard to particle sizing (based upon the intensity of scattered light) and polydispersity index (an estimate of the width of the distribution); the mean z-average diameters exhibited two peaks for the PN solutions. The principal peak had a mean intensity of 1.298±0.2 nm which represented more than 80% of the total area intensity; whereas the second peak has a widely variable mean intensity (varying from 350 to 750 nm) which was not dependant on the Ca–P concentrations (Fig. 3).

For TPN admixtures the z-average diameters (n=12) were respectively 356.0±25.4, 366.2±22.1 and 400.8±25.3 nm for N1, N2 and N3 formulas with Clinoleic®, and were respectively 264.6±22.3, 279.1±16.8 and 290.1±14.9 nm for N1, N2 and N3 formulas with Ivelip® (Table VII). In these cases, only one peak of the z-average diameters was observed, it increased with Primene® and glucose concentrations and was not influenced by the Ca and P concentrations.

Analysis of results did not point out particles diameters larger than 1 μm (about 1 to 2% of N1 samples had globule sizes between 1 and 2 μm which disappeared after successive measurement). The polydispersive index was <0.5 for TPN

Table VI. Calcium Concentrations (Expressed in mmol/l±SD) before and after Filtration (0.45 μm) of TPN (N1, N2 and N3 with 2% Ivelip®) after 24 H of Storage at 37±1°C (n=3)

Samples	P	Ca	Calcium concentrations (mmol/l)					
	Phocytan®	Ca Gluconate	Before filtration			After filtration		
N	mmol/l	mmol/l	N1 Ivelip®	N2 Ivelip®	N3 Ivelip®	N1 Ivelip®	N2 Ivelip®	N3 Ivelip®
1		30	29.80±1.82	30.25±1.52	30.38±1.72	23.48±5.27	23.68±2.23	24.89±1.99
2	40	24.5	23.92±1.90	24.40±1.71	24.31±1.11	17.98±2,29	18.54±3.24	19.35±2.27
3		15.5	14.80±1.18	15.45±1.25	15.32±1.43	11.29±2.02	11.38±0.30	12.66±1.45
4		30	29.56±1.68	30.28±2.25	30.06±1.93	24.65±2.60	22.99±2.44	25.36±2.30
5	32	24.5	24.03±1.54	24.48±2.19	24.54±1.04	20.50±1.96	18.14±1.82	17.27±1.39
6		15.5	15.16±1.08	15.55±1.25	15.13±1.35	11.02±3.10	11.85±1.21	12.52±2.16
7		30	29.43±1.91	30.14±1.82	30.15±1.82	24.91±0.79	23.44±1.45	24.23±4.22
8	26	24.5	24.33±1.73	24.55±1.63	24.46±1.61	18.81±2.95	18.14±2.19	16.15±3.48
9		15.5	15.03±1.92	15.60±1.49	15.48±1.66	11.44±0.77	12.42±1.72	13.25±2.16
Blank 1	0	30	29.74±1.49	29.40±1.97	29.87±1.53	27.47±0.53	27.22±0.28	27.19±1.03
Blank 2	0	0	0	0	0	0	0	0

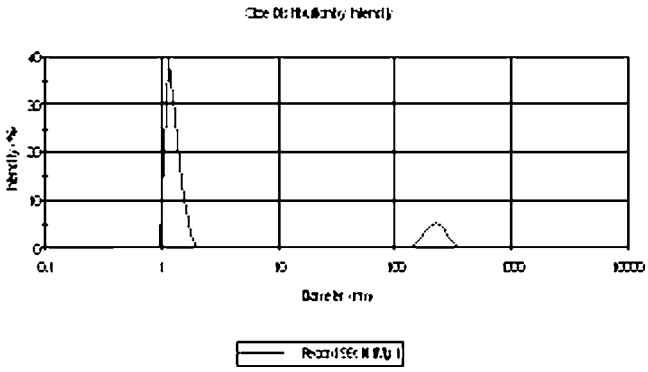

Fig. 3. Sample of the mean particle size determination of PN solutions with laser light extinction method (mean intensity of scattered light is expressed in % and corresponds to the size distribution in nm).

admixtures, which indicated a good homogeneity of the dispersion. A significant difference was found when comparing N3 to respectively N1 ($p=0.008$) and N2 ($p=0.046$) for both lipid emulsions, and it was also significant when comparing Ivelip® *versus* Clinoleic® ($p<10^{-3}$) admixtures.

The particle diameter was then checked by a Mastersizer® to detect particles greater than 6 μm. No particles greater than 6 μm were detected in neither of the experiments.

Zeta Potential Determinations

The mean values of zeta potential were not significantly different ($p=0.251$) when comparing the three TPN admixtures to each others (N1, N2 and N3) made with either Clinoleic® or Ivelip® (Table VIII).

A reduction of the zeta potential charge were observed for all samples containing various calcium and phosphate concentrations compared to the original lipid emulsion and also compared to samples without Ca–P. These later were comparable to those of the original lipid emulsion (negative charge between −40 and −50 mV), while samples with only Ca (30 mmol/l) and without P have a significant reduced zeta potential (negative charge close to −30 mV). Furthermore, higher zeta potential charges were noted for samples containing higher P concentrations (i.e. 40 mmol/l).

DISCUSSION

The precipitation of calcium and phosphate is the most likely chemically-formed precipitate to be taken into consideration in the manufacturing of TPN solutions. In the actual study using critical high amount of organic glucose-1-phosphate (G1P), the solutions remained limpid after the addition of calcium gluconate, whereas previous studies showed precipitates which appeared immediately after the addition of calcium gluconate even using very low concentrations of inorganic dibasic potassium phosphate (14).

Also, previous studies showed that Ca–P precipitation can be represented by a hyperbolic curve. It means that either by increasing calcium or phosphate concentration and keeping the concentration of the other electrolyte unchanged will have the same result on Ca–P precipitation. If the solubility product for a particular Ca–P salt is exceeded, then precipitation will occur (2,22).

Microscopic observation of the solutions after storage at 37±1°C plus one day at ambient temperature, revealed the presence of crystals with different sizes and shapes (Fig. 1A, B) in N1 admixtures (with or without lipids) that resulted probably from the precipitation of Ca with the organic phosphate G1P.

The decrease in the Ca concentration after filtration confirmed its precipitation with P, and it was more pronounced with N1 samples in which the pH was slightly more elevated than others mixtures (pH near 7).

Ca–P solubility is difficult to predict and to identify. There are many contributory factors such as temperature, time, pH, type and concentration of aminoacids, Ca and P additives, and formulation sequence. But there are some factors that can enhance the stability, such as high strength dextrose which increases mixture viscosity, and improve Ca–P solubility by restricting ionic mobility (16).

PN mixtures usually have a pH range of 5–7 at which most amino acids remain electrically neutral and exert only a

Table VII. Size Distribution (in nm±SD) of TPN Admixtures N1, N2 and N3 with Various Ca–P Concentrations Prepared with Clinoleic® or Ivelip® Lipid Emulsion and Various Concentrations of Ca and P ($n=3$)

Samples	P	Ca	Mean particle size (nm ± SD)					
	Phocytan®	Ca Gluconate	TPN samples with Clinoleic®			TPN samples with Ivelip®		
N	mmol/l	mmol/l	N1	N2	N3	N1	N2	N3
1	40	30	338.8±21.2	368.9±22.5	398.6±22.1	272.2±14.4	275.4±16.4	305.6±12.5
2	40	24.5	352.4±20.4	398.8±25.8	400.9±23.8	264.6±12.4	276.0±6.4	291.6±14.8
3	40	15.5	384.7±65.0	381.3±35.8	402.5±31.3	272.2±12.1	280.6±12.8	296.9±11.7
4	32	30	365.6±26.3	370.8±38.5	397.0±24.3	264.8±15.9	270.8±7.4	291.7±19.3
5	32	24.5	356.3±24.4	358.5±19.5	413.3±26.7	258.8±7.3	277.1±5.2	296.4±14.3
6	32	15.5	352.0±22.9	356.8±32.9	384.9±35.1	272.2±18.8	280.3±11.2	294.1±8.6
7	26	30	358.3±15.1	345.2±25.1	392.9±19.6	268.2±8.9	274.5±10.3	291.2±16.6
8	26	24.5	336.8±19.7	354.7±35.8	403.6±23.3	274.6±37.9	268.1±8.8	296.3±6.5
9	26	15.5	346.4±40.6	350.3±32.2	411.1±37.2	262.7±13.2	265.1±11.9	286.7±10.4
Blank 1	0	30	335.6±13.3	360.7±18.4	408.2±18.9	272.5±25.1	267.7±9.2	284.2±6.6
Blank 2	0	0	312.4±26.2	340.9±10.8	397.7±16.3	275.6±16.3	286.6±41.7	310.9±12.7

Table VIII. Zeta Potential (in mV±SD) of TPN Admixtures N1, N2 and N3 Prepared Respectively with Clinoleic® and Ivelip® and Various Ca–P Concentrations (n=3)

Ca–P concentrations		Zeta potential (in mV±SD)					
		TPN samples with Clinoleic®			TPN samples with Ivelip®		
P (mmol/l)	Ca (mmol/l)	N1	N2	N3	N1	N2	N3
40	30	−37.32±3.44	−35.41±4.75	−35.89±3.89	−37.16±5.97	−35.97±3.91	−36.27±2.54
40	24.5	−36.65±3.72	−34.56±5.96	−32.87±5.92	−38.70±4.26	−35.12±2.78	−34.02±1.62
40	15.5	−35.16±2.66	−38.05±3.25	−38.27±2.32	−32.90±1.79	−38.34±2.41	−35.63±3.73
32	30	−32.99±3.79	−32.86±5.32	−30.69±6.29	−37.90±1.41	−32.48±3.37	−32.46±2.33
32	24.5	−32.22±5.50	−34.30±5.46	−32.86±5.46	−37.03±2.13	−32.30±2.83	−32.86±3.54
32	15.5	−32.86±277	−33.78±5.77	−39.03±5.43	−40.88±3.41	−34.77±1.40	−36.01±4.27
26	30	−32.99±1.52	−31.36±5.26	−36.22±4.55	−32.16±2.74	−32.50±5.61	−34.70±2.15
26	24.5	−33.18±5.47	−32.67±4.41	−35.11±3.53	−32.98±3.48	−34.86±3.09	−34.78±3.47
26	15.5	−34.45±4.84	−35.54±5.36	−34.61±2.37	−33.95±1.61	−35.39±3.77	−32.55±4.13
0	30	−30.37±3.75	−30.60±5.06	−31.46±4.42	−30.48±2.12	−31.62±4.16	−31.56±3.41
0	0	−42.65±6.30	−45.20±4.15	−46.99±3.30	−42.65±4.34	−43.67±4.40	−46.39±2.74

week buffer effect. This pH also optimises the solubility of Ca–P salts.

Furthermore, all currently available IVLEs used in parenteral nutrition support have a pH ranges between 6.0 and 9.0, where the polar phosphate groups in aqueous phase of the emulsion are optimally ionised to maintain the surface charge, thus inducing a net negative charge or zeta potential between −30 and −50 mV (18). The surface potential (Zeta potential), resulting from the ionization of the hydrophilic region of the emulsifying agent on the surface of the droplet, maintains droplet separation and therefore enhances stability (21).

The pH of our admixtures ranged from 6.15±0.16 to 6.93±0.12 (with or without lipids), rising in the final pH was essentially due to the buffer effect of the G1P. Previous studies (4) showed that at physiologic pH (7.4), approximately 60% according to the Henderson Hasselbach equation, of the inorganic phosphate is in the dibasic form. Because the dibasic Ca–P salt is poorly soluble, this enhances the probability of Ca–P precipitation. By decreasing the pH by 2U, approximately 95% of the phosphate is in the monobasic form which is 60 times more soluble. However this relationship has not been described with more complex organic phosphates.

They consist of a P group covalently bounded to an organic molecule such as glycerol, glucose or fructose. The P group is not fully ionised and therefore much less available for adverse interaction with calcium; therefore precipitation of inorganic calcium phosphate should not occur (23).

In 1994, the US Food and Drug Administration (7) reported a number of clinical incidents attributed to Ca and P precipitations in patients receiving a PN solution. In all these cases, the precipitates were identified although appropriate levels of Ca and P have been used (6). This suggests that the reaction can occur spontaneously at any concentration, and challenges the accepted maximum levels at which the two electrolytes can be mixed (16). Precipitates that could not be visible to the human eye can act as a seeding point for the formation of a larger precipitate particle. The FDA alert recommends the use of filters when infusing TPN solutions through central or peripheral intravenous line. The benefits listed for in-line filters in PN solutions include removal of particulate matter, prevention of phlebitis, prevention of air embolism and sepsis. A 0.22 μm filter can be considered a sterilizing filter because it removes all particulates and all known bacteria (24). For TPN emulsions, it is recommended to use filters between 1.2 and 5 μm (25).

Low pH, high temperature, time and high concentrations of cations, such as calcium, magnesium, sodium or potassium are factors leading to destabilization of the TPN emulsion (26). Furthermore, PN admixtures intended for very young patients (i.e. neonates) have very different final composition compared to those prescribed for older children and adults due to differences in paediatric amino acid profiles (17). The use of lower pH values rapidly reduces the zeta potential, thus compromising emulsion stability. Investigators have also found that on addition of dextrose, the pH of the emulsion immediately decreased to that of the dextrose solution (27). Our results of the pH study as presented in Fig. 2 showed that N3 solutions have lower pH due to higher Primene® and glucose concentrations. Also, previous studies showed that higher glucose concentrations give a relative protection to the emulsion regarding to their zeta potential when higher Ca concentrations are used (19,20).

The pH of the amino acid used in this study Primene® was approximately 5.5, which is close to the limit of lipid destabilising pH. However, all samples were visually stable essentially due to the buffer effect of the G1P which increased the pH.

Our results corroborate with those presented previously (28), that the combinations of calcium and phosphates concentrations which do not precipitate in the lipid emulsion will not affect the mean particle diameter of the admixture after a period of time. It has been suggested that some phosphatides present in the lecithin could interact with the ions present in the aqueous phase such as calcium ions. Thus, lipid emulsions containing less lecithin concentrations could be less resistant to destabilisation involved by the cations (29).

In our experiments, filters have been partially blocked by gross lipid particles (>0.45 μm), and consequently a part of the calcium could have been captured at the fat globule surface, since the decrease of the Ca concentration in the PN

solution samples (without phosphate i.e. Blank 2) is relatively lower compared to similar TPN samples containing lipids. The significant decrease of Ca concentrations in the other samples, after storage conditions, indicated that Ca–P precipitation occurred in formulas containing G1P and it was more pronounced in samples containing the highest concentrations of both ions. The crystals (one to three per sample) observed in samples containing high calcium and phosphate concentrations and after storage conditions of the study plus one day at room temperature confirmed these results. Investigators (30) conducted a 5 μm filter analysis of 45 bags containing standard TPN formulation stored for 7 days at 4°C. Fat represented 99.4% of the filter contents, while electrolytes precipitates (Mg, Zn, Ca, Na and K) constituted less than 5%.

Furthermore, calcium ion can form bridges between two negative charges of phospholipids in the same lecithin layer or between two lipid globules (31). These bindings will immobilize the globules and will reduce the zeta potential. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating.

According to Washington *et al.* (32) a concentration of calcium between 3.5 and 4 mmol/l will neutralize the zeta potential of 20% Intralipid® emulsion. This phenomenon has been confirmed by visual observation of a cream layer followed by coalescence. Another study conducted by Chaumeil *et al.* (33) showed a more retarded destabilisation of Ivelip® emulsion in the presence of calcium between 3.5 and 7 mmol/l probably due to the presence of the sodium oleate in this product.

Two facts: one that "bridging" between the Ca cations and the negative charge phospholipid groups of the lecithin molecules induce lipid aggregations and hence instability of the emulsion. This would support the concept that an excess of cations (i.e. Ca) in the mixture reduce the zeta potential and electrophoretic mobility of the emulsion droplets, leading not only to Ca–P precipitation, but also to admixture instability. In contrast, by chelating with Ca, G-1-P has an opposite effect leading to improve stability, this was also observed previously with the use of organic fructose 1,6-diphosphate by Hicks and Hardy (16).

Our overall results suggested that the use of TPN admixtures could give a relative protection to these preparations from their physicochemical degradation. Also, the use G1P that causes a rise of the pH of the final admixture allowing the use of high concentrations of Ca without affecting the integrity of the lipid emulsion globules. Further investigations by sophisticated methods like electron microscopy and energy disperse spectroscopy are needed for particle identification (34).

CONCLUSION

The results obtained confirmed that the use of glucose-1-phosphate highly improve Ca–P solubility when used with lipid emulsions as TPN compared to PN solutions without lipids. This finding, if confirmed may represent a significant clinical advance in the care therapy by improving the availability of both Ca and P and also by eliminating the need for separate administration of lipid emulsion and its attendant risks engendered by the multiple manipulations. However, the precipitation of Ca–P could occur when organic P is used even at the daily recommended dose at critical conditions of pH, temperature and storage conditions. The risk of precipitation is higher for solutions containing 1% of Primene® with 8% glucose and lower for solutions containing 2% and 3,5% of Primene® with 14% glucose and 2% lipid emulsion.

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