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Physicochemical stability of an admixture for total parenteral nutrition

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A total parenteral nutrition (TPN) mixture is a complex chemical system designed to provide nutrients to patients unable to receive nourishment through the gastrointestinal tract. Many studies of the stability and compatibility of PN formulations are found in the literature [1–15] and the importance of the particle size distribution in these mixtures is known. In our case the TPN under study was an emulsion for intravenous (IV) administration containing lipids, amino acids, vitamins, electrolytes, major and trace elements and glucose necessary to maintain the patient's homeostasis (Table). Concentration ranges selected for the TPN under study represent those commonly used for patients receiving parenteral nutrient therapy. Some amino acids are photosensitive. The most labile amino acid is tryptophan and its deterioration is easily recognizable by the bluish or purplish tint that it develops in the presence of the antioxidant sodium bisulfite [2]. It was for this reason that tryptophan was chosen to be quantified in this work.

The stability study was carried out by storing TPN (Table) in the refrigerator (4 °C) and at room temperature (25 °C)

Table: Composition of total parenteral nutrient (TPN)

Component	Amount per 1000 ml
Amino acids[a] (g)	20
Dextrose[b] (g)	125
Phosphorus[c] (meq)	15
Sodium[d] (meq)	45
Potassium[e] (meq)	30
Magnesium[f] (meq)	5.075
Zinc[g] (mg)	2
Copper[h] (mg)	0.75
Chromium[i] (mcg)	2
Selenium[j] (mcg)	20
Molybdenum[k] (mcg)	25
Calcium[l] (meq)	3.45
Multivitamins[m] (ml)	2.5
Lipids[n] (g)	20
Chloride[d,e,i] (meq)	58.193353
Sulfate[f,g,h] (meq)	0.544824
Sterile water[o]	q.s.

[a] Amino acids 10%. Abbott Laboratories Argentina S.A., lot 29618 DM
[b] 50% Dextrose injection. Roux Ocefa S.A., Argentina, lot 034610169
[c] Sodium phosphate injection (containing phosphorus 3 mM/ml and sodium 4 meq/ml), Fada Ind. Com. and Pharm. S.R.L., Argentina, lot 1310801A
[d] Sodium chloride injection 20%, Fada lot 1591951B
[e] Potassium chloride injection (containing potassium 1 meq/ml and chloride 1 meq/ml) Roux Ocefa, lot 15603547
[f] Magnesium sulfate injection 25%, Fada, lot 1565251B
[g] Zinc sulfate (containing zinc 1 mg/ml), Fada, lot 1310311A
[h] Copper sulfate (containing copper 0.4 mg/ml), Fada, lot 1306171A
[i] Chromium chloride (containing chromium 4 μg/ml), Fada,.lot, 2008091
[j] Selenic acid (containing selenium 20 μg/ml), Fada lot 0931121
[k] Ammonium molybdate (containing molybdenum 25 μg/ml), P. L. Rivero & Cia. S.A.I.C., Argentina, lot 2/09758
[l] Calcium gluconate 10%, Fada, lot 1903131
[m] Vi Syneral I. M. V., Beta S. A., Argentina, lot 27273/68
[n] Lipids 20% injection, Abbott, lot 18431de
[o] Sterile water, Rivero, lot 601182. The amount added to each TPN solution was sufficient to bring the total to 1000 ml

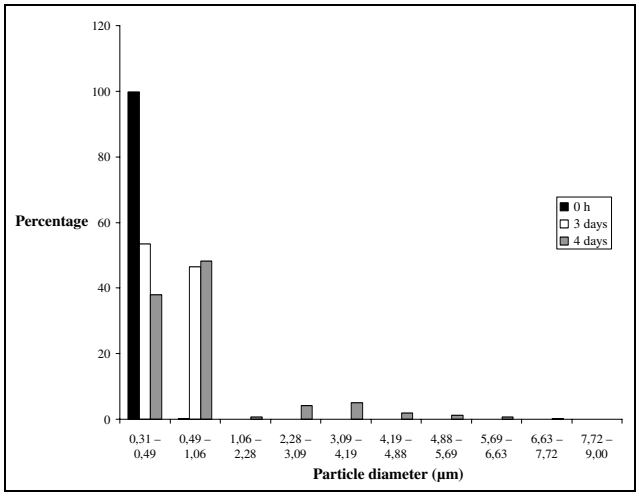

Fig.: Particle-size distribution in the TPN Lot 2, 4 °C

for eight days to determine pH, creaming, emulsion breakup, crystallization, tryptophan content by HPLC, particle size using the laser diffraction method and zeta potential. Based on the results obtained in this first stage, a second stage was carried out to determine only the factor most affected, particle size, in another TPN preparation.

Twenty bags with lipids were prepared (lot 1), of which ten were placed in the refrigerator and the rest left at room temperature, while of ten bags without lipids which were available, five were placed in the refrigerator and the rest kept at room temperature.

For the second stage of the study (lot 2) a total of twelve bags with lipids were prepared and placed in the refrigerator, while six bags without lipids were available and were also placed in the refrigerator.

All TPN mixtures stored in the refrigerator up to eight days appeared as clear, pale-yellow, free-flowing liquids with non-visible particulate matter in normal fluorescent room light. However, creaming, streaking, precipitation, and color changes were observed after keeping the sample at room temperature for eight days.

L-Tryptophan concentrations failed to change significantly during the study period. Mean pH values remained substantially unchanged from time zero up to the end of the study period. The most important change during the study was detected in the particle-size distribution of the TPN. Particles larger than 5 μm were detected at four days in the refrigerator (Fig.).

Zeta potential values of particles in suspension ranged from −40 to −47 mV, denoting that the particle surfaces are negatively charged. No significant change in the value of zeta potential was observed before the first 192 h (8 days). Particle surfaces showed a slight decrease in negative charge, as after eight days of refrigerator storage the zeta potential dropped to −37 mV and to −35 mV at room temperature.

Driscoll et al. [1] consider emulsions presenting a percentage exceeding 0.4% of particles greater than 5 μm to be unstable. Results achieved justify the final condusion: the two lots kept in the refrigerator remained stable up to the third day after preparation.

Experimental

1. Sample preparation

One liter of each TPN (Table) was prepared by aseptic technique in 1000 ml EVA (ethylvinylacetate) bags (Laboratories Grifols, Spain) and under sterile conditions in a Class 100 laminar flow cabinet, combining

automatic preparation with manual addition of nutrients, with no supplementation with drugs. An automatic Hyperformer nutrient mixer was used (Mc Gaw). The mixture was passed through a prefilter and a final 0.22 μm filter with nitrogen pressure.

2. L-Tryptophan analysis

The analysis was performed on a LiChroCART^{R} 250 · 4 mm HPLC Cartridge LiChrospher® 100 RP-18 (5 μm) Merck, coupled with a column guard of LiChroCART® 4 · 4 mm LiChrosorb® RP-18 (5 μm) Merck (Darmstadt, Germany). The mobile phase consisted of 96% solution A and 4% solution B. Solution A was 0.01 M sodium acetate buffer and solution B was acetonitrile, pH 5.45. Chromatography was performed at room temperature using a 1.8 ml/min-flow rate and a 13 min run time. Detector sensitivity was set at 0.01 a.u.f.s and eluates monitored at 254 nm. The volume of each injection was 100 μl. Under these conditions L-Tryptophan retention time was roughly 8 min. All samples containing TPN suspension were prepared as below. An accurately measured 1 ml of the TPN suspension was placed in an Altech Maxi-Clean C C18 cartridge and eluted with mobile phase to bring it to volume in a 100 ml measuring flask. This procedure was previously validated by skiping cartridges with 1 ml of standard containing an L-Tryptophan concentration similar to that of the TPN and eluting with 100 ml of mobile phase, to obtain recovery values of 97.8; 100.3; 99.6; 100,4% (mean recovery, 99.5%; SD, 1.2%; CV, 1.2%).

3. Emulsion analysis

Samples were inspected against a black background for creaming, coalescence and color change. Samples were filtered with nylon filters (1.2 μm-pore size) Micropore Separations Inc. Cat N° DDR12025SO to determine the presence of crystals.

The pH of the TPN was measured in triplicate by using an Altronix device (Model TPX). Particle size and particle size distribution of the oil droplets in the emulsion were determined by Low Angle Laser Light Scattering (Mastersizer μ, Malvern Instruments Ltd., Spring Lane South, Worcestershire WR14 1AT, UK). Electrophoretic mobilities of particles in suspension were determined in samples of parenteral solution (5 μl) diluted in 5 ml of 1 mM KCl, in a capillary H-cell with Ag/AgCl electrodes. The electrodes were connected to a direct current source fixed at 40 V. Measurements were obtained with alternating changes of the polarity of electrodes to avoid polarization. The conditions were standardized by determining the zeta potential (ξ) of a phosphatidylserine liposome whose value was roughly ±120 mV in 0.01 mM NaCl at pH 7.4. The rate of migration was determined by light microscope observation of individual particle displacement showing rectilinear and uniform movement along a reticular lattice (length 1 mm). The temperature was maintained at 25 °C in all cases. Zeta potential was calculated by the Smoluchowski equation [15].

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Mechanism of a anti-fungal action of selected cyclic dipeptides

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Cyclic dipeptides are amongst the most common derivatives found in nature and yet only few have been tested for their biological activity [1]. Previously we reported on the anti-fungal activity of cyclic dipeptides but were only able to speculate on the mechanism of action of these compounds [2]. We are now able to assess the mechanism of action of cyclo(Pro-Trp), cyclo(Trp-Pro), cyclo(Tyr-Pro), cyclo(Trp-Trp), cyclo(Phe-Phe), cyclo(Trp-Tyr), cyclo(Phe-Trp) and cyclo(Pro-Pro) on *Candida albicans*. The yeast *C.albicans* was selected as the representative of fungal pathogens as it has gained prominence as an opportunistic pathogen in AIDS patients.

Four response curves were observed that indicate that two mechanisms of action of anti-fungal activity are exhibited by cyclic dipeptides. The first mechanism is that of receptor mediation and activation of intracellular signalling and the second is that of membrane disruption. The first mechanism is effected by molecules with a negative partition coefficient (log P) (−0.31 to −2.11) [3] and those with a highly lipophilic nature (log P = 3.57) whilst the second is effected by molecules exhibiting low to increasing lipophilic nature (log P = 0.12 to 2.53). Using a transformed regression model and developing the structure – activity relationships by applying fifth order polynomials it was shown that the molecular weight of the molecules has the most significant effect on their anti-fungal activity ($r^2 = 0.998$) whilst the partition coefficient has a slightly less significant effect ($r^2 = 0.815$) [4].

The first response curve (Fig. A) is a result of those cyclic dipeptides containing an imino acid. Proline imposes conformational restraints on the peptides in which it is found [5]. Furthermore, the *cis-trans* isomerism of the *N*-alkylamide bond in the Proline containing DKPs has been implicated in the receptor mediated biological activity of proline containing compounds. This is supported by the fact that the cyclic dipeptides have a significant effect on the consumption of ATP ($r^2 = 0.869$; results not shown and [6]) and the phosphorylation of the CREB proteins ($r^2 = 0.938$; results not shown). The second response curve caused by cyclo(Trp-Tyr) (Fig. B) is found when the molecule is monofunctional. This causes the molecule to orientate itself vertically and compete with the solvent for adsorption sites. Thus cyclo(Trp-Tyr) could bind to surface or internal receptors causing the effects recorded [7].

The third response curve (Fig. C) describes concentration dependence of the activity of the cyclic dipeptides. The dipeptides causing such a response all contain only aromatic amino acids, which can participate in hydrophobic interactions [7]. The delocalized π-electron clouds that enable phenylalanine and tryptophan to interact with other π-systems and allow them to transfer electrons may allow the disruption of the negative charge maintained on biological membranes. The final response (Fig. D) is caused by the hydrophobic cyclo(Phe-Phe) due to the increasing difficulty of the dipeptide to find a vacant binding site on the *C.albicans* surface. Furthermore, the hydrophobic nature of these dipeptides would suggest that cyclo(Phe-Phe) would interfere with plasmamembrane integrity [8].