Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 6, 2015

Original Article

GAMMA RADIATION AS A METHOD FOR STERILIZATION OF ALL-IN-ONE ADMIXTURES BAGS FOR CLINICAL USE: A STUDY OF STABILITY

CRISTIANA BARBOSA SILVA[1], THIAGO DIAS DE VASCONCELOS ARAUJO[1,2], PAULO ROBERTO LEITAO VASCONCELOS[1,3], HAROLDO CESAR BESERRA DE PAULA[1,4], NADIA ACCIOLY PINTO NOGUEIRA[1,5], CARLOS ALBERTO BRAYNER DE OLIVEIRA LIRA[6], GISELE MEDEIROS BASTOS[5], DAVID JOHN WOODS[7], MARTA MARIA DE FRANCA FONTELES*[1,2]

[1]Federal University of Ceará, Brazil, [2]Faculty of Pharmacy, Dentistry and Nursing, Research Laboratory in Clinical Pharmacy and Translational, [3]Faculty of Medicine, Experimental Surgery Laboratory, [4]Faculty of Chemistry, Physicochemical Laboratory, [5]Faculty of Pharmacy, Dentistry and Nursing, Applied Microbiology Research Laboratory, [6]Federal University of Pernambuco, Department of Nuclear Energy, [7]School of Pharmacy, University of Otago and Best Practice Advocacy Centre, Dunedin, New Zealand
Email: martafonteles@yahoo.com.br

Received: 18 Mar 2015 Revised and Accepted: 14 Apr 2015

ABSTRACT

Objective: The aim of this work was to evaluate the stability of all-in-one (AIO) admixtures exposed to gamma irradiation sterilization.

Methods: The samples were divided into four groups with 10 bags each: a) Group I: control samples (bags without sterilization or inoculation with microorganisms); b) Group II: bags sterilized by gamma irradiation; c) Group III: bags inoculated and then irradiated and, d) Group IV: bags only inoculated. The following studies were performed: macroscopic analysis of admixtures; physicochemical stability; degree of lipoperoxidation (LPO), and microbiological tests.

Results: Gamma irradiation sterilization was 100% effective, since no irradiated sample showed growth of microorganisms. All groups exhibited similar particle size distribution, but a longer storage time led to a smaller percentage of large particles. In general, irradiated samples showed reduced LPO.

Conclusion: Gamma irradiation sterilization of these admixtures can be extended to clinical practice, as it results in physicochemically stable admixtures.

Keywords: Total parenteral nutrition, Gamma irradiation, Physicochemical stability, Lipid peroxidation, Microbiological analyses.

INTRODUCTION

The various products used to compound all-in-one (AIO) admixtures or Total Parenteral Nutrition (TPN) can interact by various mechanisms, some of which are not completely understood. These interactions can cause physicochemical instability requiring strict control during production and clinical use [1].

The most critical property used to determine lipid emulsion physicochemical stability is the percentage of particles larger than 5 µm. This must not exceed a total of 0.05% of lipid globules in admixture, according to United States Pharmacopoeia (USP) specifications, because lipid globules of this size may cause embolic syndrome by obstruction of the pulmonary capillaries, mainly in preterm infants [2–4].

Lipid peroxidation (LPO), which occurs in the presence of reactive oxygen species (ROS), is another property that can compromise the stability of the admixture [1]. LPO is a process under which antioxidants such as free radicals, or nonradical species attack lipids, resulting in lipid peroxyl radicals and hydroperoxides, which trigger oxidative stress and toxicity. Polyunsaturated fatty acids (PUFAs) are more susceptible to lipid peroxidation than other fatty acids [5].

LPO is influenced by the composition of the lipid emulsion, the storage conditions (temperature, light), and by the plastic used in the manufacture of the infusion bags [1]. The process of LPO can be reduced when photo-resistant infusion devices are used and when the TPN is protected from light, stored under refrigeration and packed into bags impermeable to O_2 [6].

Multilayered bags are preferable for packaging all-in-one admixtures as they protect against the adsorption of oxygen, preventing LPO and increasing the stability of the admixture [7].

The literature reports that various micro-organisms may grow using the nutrients in TPN, for example: *Candida albicans*, *Staphylococcus saprophyticus*, *Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis*. What determines which micro-organism will grow is the proportion of nutrients in TPN [8]. Species of *Enterobacter* are important nosocomial pathogens. They have been associated with intravenous use of contaminated products, due to their ability to grow in intravenous solutions. The use of parenteral nutrition is a risk factor for *Enterobacter* infection [9].

Gamma irradiation using cobalt-60 has been cited to be an effective method of sterilization for TPN admixtures without compromising physicochemical stability [10]. In order to confirm this context, the present work aimed to evaluate the stability of TPN infusions submitted to gamma irradiation through macroscopic analysis of the AIO admixtures, analysis of physicochemical parameters of stability and quantifying the degree of LPO. The sterility of the irradiated admixtures was assessed by microbiological testing of representative samples of TPN bags prepared in the northeast of Brazil.

MATERIALS AND METHODS

Preparation of samples and experimental groups

TPN bags (samples: n=40) were prepared according to local Guidelines for Good Practice for Preparation of Parenteral Nutrition, with standardized formulations, routinely used to supply the nutritional needs of adults (table 1). The samples were prepared asseptically, in a clean room (controlled environment area with filtered air) using a class 100 Horizontal Laminar Flow Cabinet (Veco ®) situated in a private institution in Fortaleza, Ceará, Brazil. The samples were divided into four groups with 10 bags each: a) Group I: control samples (bags without sterilization and without inoculation); b) Group II: bags sterilized by gamma irradiation; c)

Group III: bags inoculated with microorganisms and then sterilized by gamma irradiation and, d) Group IV: bags only inoculated. All bags were subjected to a macroscopic observation, including the tendency for cream formation and color change.

From the 10 bags in each group, five were stored under room-controlled temperature (22 ° to 28 °C) and the others were stored at refrigerated controlled temperature (2 ° to 8 °C). All samples were analyzed at time zero (T_0), 24, 48, 72 and 168 hours (h) after preparation. At each time point, 3 ml aliquots were aseptically withdrawn from each bag at the specified time to perform each of the tests. Physicochemical parameters of stability were quantified (mean particle size of lipid droplets and their distribution) as well as the degree of LPO. Microbiological tests were conducted to assess the sterility of the samples.

Statistical analysis (ANOVA) was performed using Prism version 5.0, with the significance level set at *P<0.05. This study was approved by the Ethics Committee of the Federal University of Ceará (Process number 142/07).

Table 1: Composition of standardized bags for adult patients

Components	Quantity
Standard Amino Acids Solution*	50.0 g
Glucose Solution 50%	125.0 g
Lipid Emulsion MCT/LCT 20%**	50.0 g
Sodium Chloride Solution 20%	34.0 mEq
Potassium Chloride Solution 10%	33.0 mEq
Magnesium Sulfate Solution 50%	5.0 mEq
Calcium Gluconate Solution 10%	5.0 mEq
Potassium Phosphate Solution 2 mEq/ml	5.0 mEq
Trace Elements***	1.0 ml
Vitamin C[1]	5.0 ml
B-Complex Vitamins[2]	4.0 ml
Total Volume	1058.3 ml

Source: Formulations of parenteral nutrition standard for adults, adapted [11]

*Polyaminoacid Solution 10% (w/v), composition (100 ml): L-isoleucine 5.00 mg; L-leucine 7.40 mg; L-lysine 6.60 mg eq. A L-lysine 9.31 mg; L-methionine 4.30 mg; L-fenilanine 5.10 mg; L-trionine 4.40 mg; L-tryptophan 2.00 mg; L-valine 6.20 mg; L-arginine 12.0 mg; L-histidine 3.00 mg; glicine 14.0 mg; L-alanine 15.0 mg; L-proline 15.0 mg; glacial acetic acid 8.01 mg; sodium hydroxide; water for injection; Aminoacid total 100 g/l; Nitrogen total 16.4 g/l. **Lipid emulsion 20%, Composition (100 ml): Long-chain triglycerides (soya oil) 10.0 g; medium-chain triglycerides 10.0 g; egg lecithin 1.20 g; glycerol 2.50 g; water for injection. ***Trace elements solution for adult use, composition (1 ml): zinc 2.5 mg (zinc sulphate $7H_2O$); copper 0.8 mg (copper sulphate $5H_2O$); manganese 0.4 mg (manganese sulphate H_2O); chrome 10 µg (chome chloride $6H_2O$); water for injection. [1]Composition (5 ml): vitamin C 500 mg; vehicle (sodium bisulphite, sodium hydroxide, water for injection); [2]Composition (2 ml): thiamine chloride (B_1) 8 mg, riboflavin (B_2) 2 mg, pyridoxine chloride (B_6) 4 mg, nicotinamide (PP) 40 mg, D-pantenol (pro-vitamin B_5) 6 mg, vehicle (sodium chloride, phenol, water for injection).

Inoculation of samples

Before the gamma irradiation (in case of Group III), samples from Group III and IV were inoculated with microorganisms at time zero (T_0). The microorganism used in the experiment (*Enterobacter cloacae*) was incubated in BHI broth (Brain Heart Infusion) at 35–37 °C for 24 h. Bacterial suspensions were then prepared in saline 0.85% to obtain moderate turbidity visibly equivalent to 0.5 on the McFarland scale which equates to a concentration of about 10^8 CFU/ml. Subsequently, decimal serial dilutions were prepared using 9 ml of saline solution to each 1 ml of the previous dilution [12] to give final concentrations of 10^8, 10^7, 10^6, 10^5 and 10^4 CFU/ml.

The bags of TPN were then inoculated, using aseptic technique, with 1 ml of each concentration. Following homogenization of the admixture, 3 ml aliquots were immediately withdrawn for analysis (the others aliquots withdrawn in subsequent steps and tests were also 3 ml). That point, after inoculation, was considered as time zero (T_0). The other counts were made at times: 24, 48, 72 and 168 h.

Irradiation of samples

Only the samples from Groups II and III were individually sterilized by gamma radiation, using a Gamma Irradiator (source Cobalt-60), model Gamma Cell® 220 Excel from MDS Nordion, and a radiation dose of 1.5 kGy. The Department of Nuclear Energy of Federal University of Pernambuco made this equipment available for our study.

Before irradiating the 20 bags from Groups II and III, aliquots were withdrawn to perform the analysis at time zero (T_0). At the same time, aliquots were withdrawn from the other 20 bags (Groups I and IV).

Throughout the period in which the samples from Groups II and III were being transported and irradiated, the other samples from Groups I and IV remained under the same conditions (refrigerated controlled temperature: 2 ° to 8 °C).

After the irradiation process, half of samples from all groups were stored at room-controlled temperature (22 ° to 28 °C) and the other half was stored under refrigerated controlled temperature (2 ° to 8 °C). Subsequently, aliquots were withdrawn from all bags for analysis at each time point: time zero (T_0), 24, 48, 72 and 168 h, as described by Sforzini and colleagues [13] and Antunes [14]. All samples were stored protected from light throughout [15].

Analysis of samples

Physicochemical stability tests

The tests to evaluate physicochemical stability were performed in duplicate at all time intervals (T_0 and 24 to 168 h), using a particle analyzer model Zetasizer Nano® series Nano ZS® of Malvern Instruments Ltd. This equipment is able to measure the characteristics of particles in liquid environments, within a range of particle size varying from 0.4 nm to 10 µm. The device determines particle size and distribution through a process called Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy (PCS), which measures Brownian Motion (particulate dispersion movement), linking it to the size of the particles [16, 17].

At each time interval an aliquot was withdrawn from each bag. Then, into a cuvette with 1 ml of distilled water, one drop of this sample was added (just enough to make the solution slightly turbid according to MALVERN INSTRUMENTS LTD [18]). Then, the cuvette was put into the device for examination.

Tests of lipoperoxidation

The degree of LPO of the sample was measured using the dosage of thiobarbituric acid reactive substances-TBARS assay, according to the method described by Agar [19]. Tests were performed in duplicate at all time intervals. Aliquots were withdrawn aseptically from each bag and 250 µl of this aliquot was added to an Eppendorf reagent reservoir in a water-bath at 37 °C for 1 h. Then, 400 µl of perchloric acid 35% was added and centrifuged for 20 min at 14 000 RPM. Next, 200 µl of thiobarbituric acid 1.2% was added to the Eppendorf in a water-bath at 90-100 °C for 30 min. Finally, the reservoir was cooled in an ice bath prior to reading at 532 nm in a spectrophotometer.

Microbiological tests

The analysis to count viable microorganisms used the method of depth spreading or plating (per plate) [12]. The procedure, in duplicate, was performed at each time interval of the study: T_0 (inoculation), 24, 48, 72 and 168 h.

A 3 ml aliquot was withdrawn from each bag, but only 1 ml was added to a sterile Eppendorf. Then, Triton X-100 10% was added to facilitate homogenization of the sample with the growth medium. The homogenate was added to an empty sterile plate (90x15 mm).

The growth medium (Agar Plate Count) in liquid form at about 40–45 °C, was poured into the plate sufficient to cover the base (12–15

ml) with light rotational movements for homogenization. The plates were incubated for 48 h in a bacteriological stove regulated at 35–37 °C. After 48 h of incubation, the colonies, which grew both on the surface of agar and into the agar, were counted using an electronic colony meter. The result was expressed in CFU/ml.

RESULTS

Macroscopic properties

Formation of cream

Cream formation was more apparent in samples stored at room-controlled temperature (22 ° to 28 °C). In these samples a cream was observed, due to accumulation of triglyceride particles on the surface of the formulation, giving the appearance white regions on the admixtures' surface.

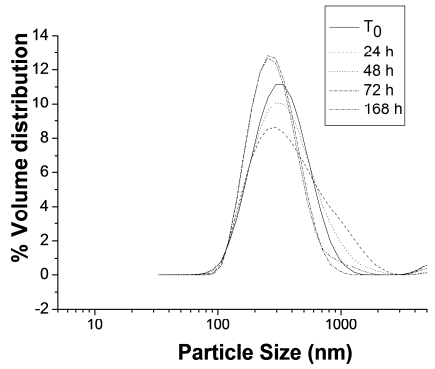
The formation of cream occurred gradually over time, and was initially found 72 h after preparation. In the irradiated samples (Groups II and III), cream formation was much less apparent at both storage temperatures and was noticed only at the end of the study. Cream formation was also noticed in bags that were not irradiated and kept under refrigeration.

Change of color

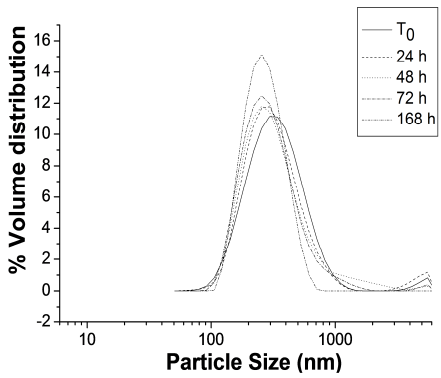
The irradiated samples stored at room temperature and refrigeration (Groups II and III) did not show any change in color from T_0 until the end of the study. Similarly, the bags stored under refrigeration (2 ° to 8 °C) without irradiation (Groups I and IV), also showed no color change from T_0 up until the end of the study period (168 h). Only the bags stored under room temperature of Groups I and IV showed color change, gradually during the analysis time.

Physicochemical stability test

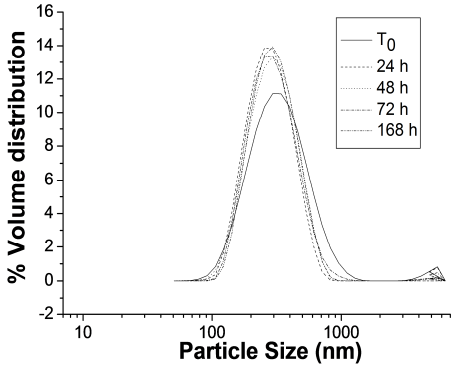
The particle size ranged from 37.8 nm to 5560 µm for all groups investigated. The average size was in the range 255–395 nm, corresponding to 11.78–15.14% of volume distribution. After 24 h of sample preparation, it appears that molecule rearrangements occur, leading to a narrower volume distribution, with a smaller average particle size. This behavior was exhibited by all sample groups, independent of storage temperature or irradiation status (fig. 1). It should be noted that all groups exhibited similar particle size distribution, for particle size larger than 5 µm, at room and refrigerated temperatures, whereas longer storage time leads to a smaller percentage of large particles, as it can be seen in fig. 2.



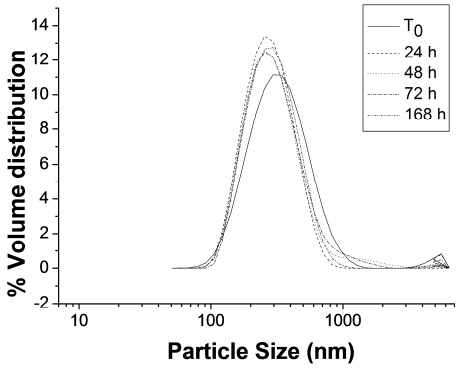
a



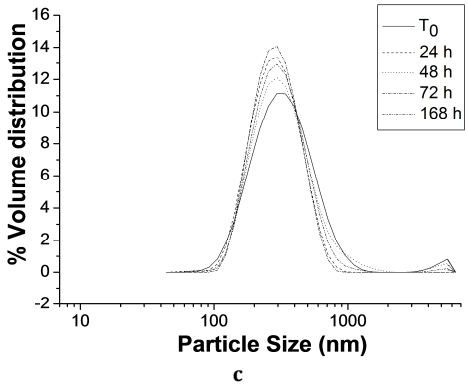
a1



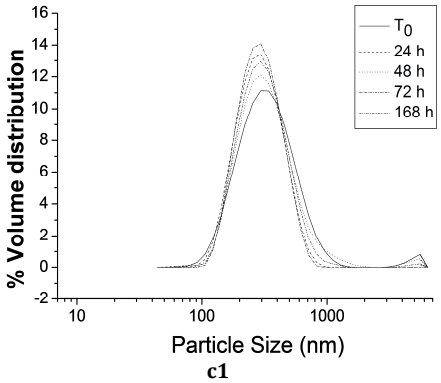
b



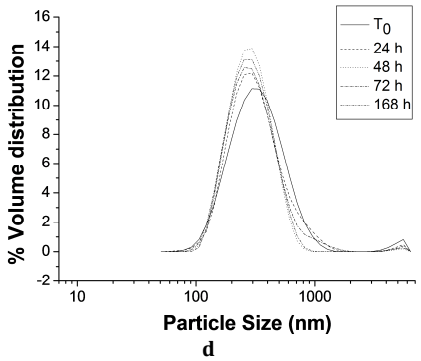
b1



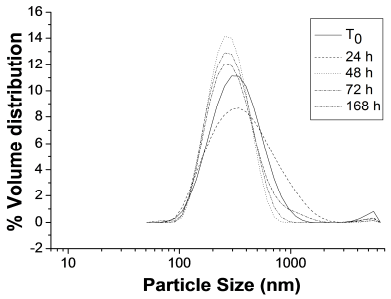
c



c1



d



d1

Fig. 1: Size and lipid particle distributions for samples of Groups I-IV, at room-and refrigerated controlled temperature, at different storage times a, a1: Group I–control samples (bags without sterilization and without inoculation); b, b1: Group II-bags sterilized by gamma irradiation; c, c1: Group III-bags inoculated with microorganisms and then sterilized by gamma irradiation; d, d1: Group IV-bags only inoculated. Left side: room temperature; Right side: refrigerated temperature

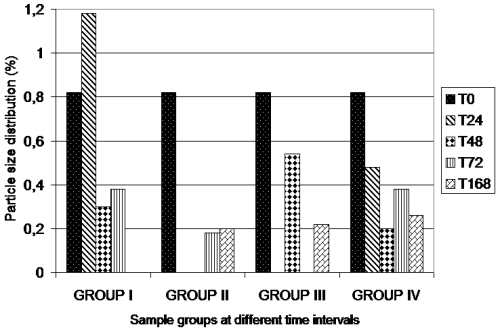


Fig. 2: Lipid size distribution for particles larger than 5 µm of all Groups, at room temperature
Group I–control samples (bags without sterilization and without inoculation); Group II-bags sterilized by gamma irradiation; Group III-bags inoculated with microorganisms and then sterilized by gamma irradiation; Group IV-bags only inoculated

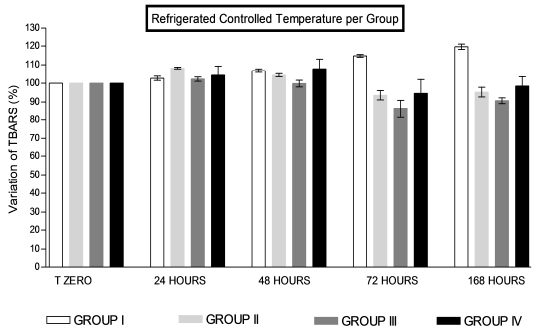


Fig. 4: Variation in percentage of TBARS (thiobarbituric acid reactive substances) in samples under refrigerated controlled temperature (2 ° to 8 °C) Group I–control samples (bags without sterilization and without inoculation); Group II-bags sterilized by gamma irradiation; Group III-bags inoculated with microorganisms and then sterilized by gamma irradiation; Group IV-bags only inoculated

Lipoperoxidation stability test

The levels of LPO remained higher in the bags stored at room-controlled temperature (22 ° to 28 °C) (fig. 3) than in those under refrigerated temperature (2 ° to 8 °C) (fig. 4) (*P<0.05). The lowest levels of LPO occurred in the irradiated samples with even a reduction in LPO at some time intervals compared with the respective controls (Group I).

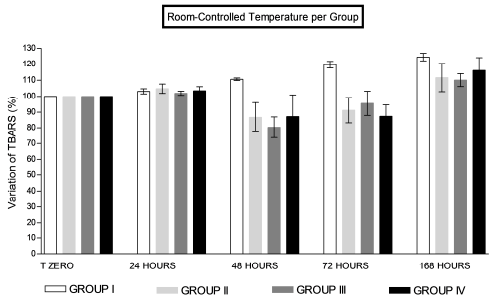


Fig. 3: Variation in percentage of TBARS (thiobarbituric acid reactive substances) in samples under room-controlled temperature (22 ° to 28 °C) Group I–control samples (bags without sterilization and without inoculation); Group II-bags sterilized by gamma irradiation; Group III-bags inoculated with microorganisms and then sterilized by gamma irradiation; Group IV-bags only inoculated

Microbiological test

Samples of Group I (control samples) showed growth of microorganisms 168 hours after preparation. The samples stored under room-controlled temperature showed more microbial growth. Samples of Group II (irradiated only) showed no growth of microorganisms during the study period. Samples of Group III (samples inoculated and irradiated) showed growth of microorganisms only in the analyses performed at T_0 (after the inoculation of microorganisms); after sterilization by gamma radiation there was no further growth. Group IV (samples only inoculated) showed growth of microorganisms at all time intervals. It was also observed that the quantity of microorganisms decreased over time.

DISCUSSION

In relation to macroscopic properties of the samples, the first visible sign of change was the formation of cream, a dense layer on the surface of the AIO admixture. A cream layer is a normal alteration of this pharmaceutical formulation. Some studies report that the formation of cream can occur without significant change in the lipid particle size, and the AIO admixture can be administered if the lipid is dispersed by light homogenization, since AIO admixtures are generally safe for patient administration, because it is a reversible condition [1, 6, 20, 21].

Our samples at room-controlled temperature showed more pronounced cream formation, similar to that reported in other studies [22-24]. In fact, according to Driscoll *et al.* [22] admixtures made with only LCT-based lipid emulsion (long chain triglycerides)

showed visible signs of instability after only 30 h at room temperature. Lobo and colleagues [23] found a cream layer formation from 48 h. In addition, Ribeiro *et al.* obtained results that ranged from 48 to 72 h at room temperature, depending on the amount of calcium in the formulation [24].

Free oil formed as a result of phase separation. Driscoll and colleagues [22] also reported that admixtures composed with the MCT/LCT-based lipid emulsion (medium chain and long chain triglycerides), remained stable for long, which also occurred in our study, since the first signs of cream formation were observed at 72 h after the admixture preparation. Furthermore, the results obtained by other studies at refrigerated controlled temperatures [23-25] reported creaming formation at similar times those observed in our study.

Driscoll and colleagues [22] also identified that for every 10 °C increase in temperature, there was a two–three fold increase in the degradation rate of lipid emulsions in AIO admixtures. Therefore, a difference of about 20 °C between the refrigerated and the room temperature is significant to cause destabilization. However, storage under refrigeration only prolongs the inevitable process of destabilization, which is a characteristic of the AIO admixture. Admixtures containing a MCT/LCT-based lipid emulsion are easier to homogenize because the size of the triglyceride chain is reduced and the interfacial tension between the oil and water phases is lowered conferring better admixture stability.

The irradiated samples showed an almost imperceptible formation of cream, which occurred later in the study in samples stored at both temperatures when compared to non-irradiated admixtures. A study of Du Plessis & Rosekilly [10] of gamma irradiation sterilization applied to AIO admixtures reported similar results, in which neither formation of cream nor phase separation was observed in the irradiated admixtures, even in samples irradiated at doses above 8.3 kGy. Irradiation did not affect the physical stability of the lipid component in AIO admixtures.

The change of color occurred in the non-irradiated admixtures stored under room-controlled temperature. This is an undesirable change, because it can be a cause for loss of some nutrients. The color alteration may be due to Maillard's reaction, also known as non-enzymatic browning, which is due to a series of chemical reactions involving amino acids and carbohydrates such as glucose. Several factors such as high temperatures, exposure to light and time of contact between the components of the admixture, favor non-enzymatic browning [6, 26, 27].

In our work, all bags containing the admixtures were protected from light (light is another factor that may cause color alteration of the admixture [24]), except at the time when the aliquots were withdrawn for the tests. On the other hand, some bags were stored under room-temperature, where the time of contact between the components of the admixture at higher temperatures is relatively long. Such samples suffered Maillard's reaction, thus explaining the color change.

The change of color may also be due to the degradation of some vitamins added to AIO admixtures, especially vitamin C, which changes color to yellow or brown due to hydrolysis, and the B complex vitamins, especially vitamin B_1, which changes color due to the formation of thiochrome [6, 26]. Although studies that correlate gamma radiation use and the color change in AIO admixtures were not found, our results suggest that the use of gamma radiation preserves the color, which may indicate conservation of vitamins and nutrients which can undergo Maillard's reaction.

In relation to physicochemical stability, the average size of lipid particles (255–395 nm) are in agreement with USP 31<729>, which indicates that the mean droplet diameter size obtained by light scattering methods must be less than 500 nm for lipid injectable emulsions [3, 28].

Lipid particles>5 µm were present in all groups at time zero. Samples of Group I, stored in room temperature, showed the highest percentage of lipid particles>5 µm, reaching 1.18% in the samples analyzed at 24 h. In bags stored under refrigeration, the percentage of lipid particles>5 µm reached 0.78%, also within 24 h. The same occurred in samples from Group IV. Similar results were observed by Driscoll *et al.* [29], who found the percentage of lipid particles>5 µm higher than 0.4% between 6 and 24 h after preparing the admixture. However, Lobo *et al.* [23] did not observe any formulation with percentage of lipid particles>5 µm, at refrigerated temperature and at room temperature, for all storage times.

Although administration of TPN occurs within 24 h of preparation, such admixtures are already unsuitable for use. In other studies, Driscoll [17, 29] reported that admixtures containing lipid particles>5 µm, at a percentage higher than 0.4% of total fat, become unstable and are unfit for human administration. Lipid particles higher than 5 µm may clog the lung capillaries, which can cause embolism and other complications including death [3, 30].

Studies by Driscoll & Lobo [22, 23, 31] reported a reduction in the percentage of lipid particles>5 µm over time, which the authors attributed mainly to extensive damage (separation of phases) of lipid emulsion, resulting in a migration of larger particles to the surface of the admixture, making it non-homogeneous. In these same studies, the authors concluded that destabilization of emulsions occurs at some point between 8 and 24 h after preparation and that in all lipid emulsions, lipid particles>5 µm were found at all time intervals.

In Groups I and IV, a gradual reduction in the percentage of lipid particles>5 µm over time was also observed, reaching zero at 168 h for the samples of Group I stored at room-controlled temperature.

The samples of Groups II and III also showed lipid particles>5 µm just after preparation (T_0). However, in the analysis performed after irradiation, lipid particles>5 µm were no longer found. This occurred in the samples stored in room temperature and in the refrigerated samples.

Du Plessis & Rosekilly [10] found similar results. No sample contained lipid particles>5 µm after irradiation, even when higher doses, up to 8.3 kGy, were applied. Our study used a dose of 1.5 kGy. This result suggests that the gamma irradiation sterilization makes the AIO admixtures safer regarding the risk of embolism.

In the LPO studies, the control samples stored at both temperatures showed a gradual increase in TBARS over time, being less pronounced in the refrigerated samples. When samples from all groups were compared, it was observed that levels of LPO remained higher in the bags stored in room-controlled temperature (22 ° to 28 °C) than in those under controlled refrigeration (2 ° to 8 °C). Steger & Mühlebach [15] report that an increase in storage temperature results in a significant increase in LPO of AIO admixtures and that each 10 °C increase in temperature results in an incremental increase in LPO.

Sobotka [1] and Picaud *et al.* [32] reported exposure to light significantly increase LPO. Sobotka also stated that use of multilayered bags (less permeable to oxygen), protection from light and storage under refrigeration, reduces the infusion of peroxides to patients. In the present study, the samples were protected from light and packaged into multilayered bags.

In our work, the irradiated samples generally showed a reduction in TBARS. No reports of the occurrence of LPO related to gamma radiation sterilization of admixtures were found. However, a study of Badr [33] reports that gamma irradiation, at a dose of 3 kGy, had no significant effects on the fatty acid profile of lipids from egg yolk, and that irradiation, at room temperature, followed by storage at refrigeration (4 °C±1 °C), seems to be a suitable treatment for egg white and yolk. The author also reports that irradiation of egg yolk caused an increase in the content of free fatty acids, increasing the LPO component of the egg yolk, but such increase was relatively low and within acceptable standards.

Tükenmez *et al.* [34] found that gamma irradiation applied in the presence of oxygen accelerates the self-oxidation of lipids in fish, and the presence of oxygen greatly influences the apparent value of oxidation produced, but that this may be linked to the properties of the food and the conditions of processing, such as the radiation dose

applied, temperature and packaging. Kfouri Filho & Akamine [3] mentioned that high temperatures increase the activation energy of the molecules, and the degradation reactions (oxidation and hydrolysis) are more likely to occur at higher temperatures.

In the samples that were inoculated only, stored at 22 ° to 28 °C, there was an increase in the TBARS 24 h immediately after preparation, but after inoculation there was a reduction, which remained until 72 h, followed by a considerable increase at 168 h. In refrigerated samples there was an increase in TBARS up to 48 h, followed by a reduction at 72 h and then another increase at 168 h. After inoculation, the microorganisms possibly inactivated the peroxides, due to their catalase-positive characteristic, which is peculiar to *Enterobacter cloacae* [35]. With time, the supply of food for the microorganisms is exhausted, causing their death and allowing a further an increase in LPO reflected by an increase in TBARS at the end of the study.

In the samples only irradiated, microorganisms did not grow in any analysis. The same occurred in the samples inoculated and irradiated, in which even bags with the highest inoculums (10^8 CFU/ml), gamma irradiation was effective in destroying all the microorganisms.

A study mentioned that *Enterobacter cloacae* is sensitive to radiation at a dose of only 0.40 kGy [10]. It also reports that even with the highest concentration of microorganisms inoculated (7.6×10^4 CFU/ml) in admixture, and the lowest dose of irradiation applied (1.5 kGy), no microorganisms were detected, indicating the effectiveness of the gamma irradiation in sterilizing AIO admixtures.

In Group IV, microorganisms grew in all samples, since these admixtures were inoculated with microorganisms, and were not irradiated. However, over time, the counts of these microorganisms decreased, which may have occurred due to both a shortage of food and pH reduction caused by an increase in LPO over time [36]. Sobotka [1] describes that a low pH of around 5.5 prevents the growth of microorganisms in AIO admixtures.

CONCLUSION

Gamma radiation sterilization at a dose of 1.5 kGy, applied to AIO admixtures was 100% effective, since no irradiated sample showed growth of microorganisms, even with the highest inoculum of bacteria, suggesting that this dose seems sufficient to sterilize the samples.

As irradiated admixtures demonstrated physicochemical stability (obtaining good results with respect to creaming, color change and particle size), there is potential for their use in clinical practice. Further studies are required to confirm these findings.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Sobotka L. Bases da Nutrição Clínica. 3rd ed. Rio de Janeiro (RJ): Rubio; 2008.
2. Driscoll DF. Lipid injectable emulsions: Pharmacopeial and safety issues. Pharm Res 2006;23(9):1959-69.
3. USP General chapters. Globule size distribution in lipid Injectable emulsions. USP31/NF26; 2008. p. 285–7.
4. Hill SE, Heldman LS, Goo ED, Whippo PE, Perkinson JC. Fatal microvascular pulmonary emboli from precipitation of a total nutrient admixture solution. J Parenter Enteral Nutr 1996;20(1):81-7.
5. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longevity 2014;8:360-438.
6. Kfouri Filho M, Akamine D. Terapia Nutricional Parenteral. 2nd ed. São Paulo (SP): Atheneu; 2005.
7. Mühlebach S. Practical aspects of multichamber bags for total parenteral nutrition. Curr Opin Clin Nutr Metab Care 2005;8(3):291-5.
8. Zingg W, Tomaske M, Martin M. Risk of parenteral nutrition in neonates–an overview. Nutr 2012;4(10):1490-503.
9. Sanders Jr WE, Sanders CC. Enterobacter spp: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev 1997;4(2):220-41.
10. Du Plessis TA, Rosekilly IC. The radiation enhancement of the sterility assurance levels of sterile fluids–A case study. Radiat Phys Chem 1995;46(4-6):611-5.
11. Waitzberg DL. Nutrição oral e parenteral na prática clínica. 4th ed. São Paulo (SP): Atheneu; 2009.
12. Silva N, Junqueira VCA, Silveira NFA. Manual de métodos de análise microbiológica de alimentos. 4th ed. São Paulo (SP): Varela; 2010.
13. Sforzini A, Bersani G, Stancari A, Grossi G, Bonoli A, Ceschel GC. Analysis of all-in-one parenteral nutrition admixtures by liquid chromatography and laser diffraction: study of stability. J Pharm Biomed Anal 2001;24(5-6):1099-109.
14. Antunes MS. Estudo à microscopia eletrônica da estabilidade física de emulsões lipídicas em soluções nutritivas parenterais. Rev Bras Farm 2004;85(3):121-7.
15. Steger PJK, Mühlebach SF. *In vitro* oxidation of i. v. lipid emulsions in different all-in-one admixture bags assessed by an iodometric assay and gas-liquid chromatography. Nutr 1997;13(2):133-40.
16. Juamaa M, Müller BW. The stabilization of parenteral fat emulsion using non-ionic ABA copolymer surfactant. Int J Pharm 1998;174(1-2):29-37.
17. Driscoll DF, Bacon MN, Bistrian BR. Physicochemical stability of two types of intravenous lipid emulsion as total nutrient admixtures. J Parenter Enteral Nutr 2000;24(1):15-22.
18. Zetasizer Nano Series User Manual. Worcestershire: Malvern Instruments Ltd; 2004.
19. Agar E, Bosnak M, Amanvermez R, Demir S, Ayyildiz M, Celik C. The effect of ethanol on lipid peroxidation and glutathione level in the brain stem of rat. NeuroReport 1999;10(8):1799-801.
20. Discoll DF. Total nutrient admixtures: theory and practice. Nutr Clin Pract 1995;10(3):114-9.
21. Gonyon T, Patel P, Owen H, Dunham AJ, Carter PW. Physicochemical stability of lipid injectable emulsions: correlating changes in large globule distributions with phase separation behavior. Int J Pharm 2007;343:208-19.
22. Driscoll DF, Giampietro K, Wichelhaus DP, Peterss H, Nehne J, Niemann W, *et al.* Physicochemical stability assessments of lipid emulsions of varying oil composition. Clin Nutr 2001;20(2):151-7.
23. Lobo BW, da Veiga VF, Cabral LM, Michel RC, Volpato NM, de Sousa VP. Influence of the relative composition of trace elements and vitamins in physicochemical stability of total parenteral nutrition formulations for neonatal use. Nutr J 2012;11:26.
24. Ribeiro Dde O, Lobo BW, Volpato NM, da Veiga VF, Cabral LM, de Sousa VP. Influence of the calcium concentration in the presence of organic phosphorus on the physicochemical compatibility and stability of all-in-one admixtures for neonatal use. Nutr J 2009;8:51.
25. Lee MD, Yoon JE, Kim SI, Kim IC. Stability of total nutrient admixtures in reference to ambient temperatures. Nutr 2003;19(10):886-90.
26. Stegink LD, Freeman JB, Den Besten L, Filer LJ Jr. Maillard reaction products in parenteral nutrition. Prog Food Nutr Sci 1981;5(1-6):265-78.
27. Rigo J, Senterre J. Nutritional needs of premature infants: Current issues. J Pediatr 2006;149(5):80-8.
28. Driscoll DF. The pharmacopeial evolution of intralipid injectable emulsion in plastic containers: from a coarse to a fine dispersion. Int J Pharm 2009;368(1-2):193-8.
29. Driscoll DF, Nehne J, Peterss H, Franke R, Bistrian BR, Niemann W. The influence of medium-chain triglycerides on the stability of all-in-one formulations. Int J Pharm 2002;240(1-2):1-10.
30. Carpentier YA. Intravascular metabolism of fat emulsions: the Arvid Wretlind Lecture, ESPEN 1988. Clin Nutr 1989;8(3):115-25.
31. Driscoll DF, Nehne J, Peterss H, Klütsch K, Bistrian BR, Niemann W. Physicochemical stability of intravenous lipid emulsion as

all-in-one admixtures intended for very young. Clin Nutr 2003;22(5):489-95.

32. Picaud JC, Steghens JP, Auxenfans C, Barbieux A, Laborie S, Claris O. Lipid peroxidation assessment by malondialdehyde measurement in parenteral nutrition solutions for newborn infants: a pilot study. Acta Paediatr 2004;93(2):241-5.
33. Badr HM. Effect of gamma radiation and cold storage on chemical and organoleptic properties and microbiological status of liquid egg white and yolk. Food Chem 2006; 97:285-93.
34. Tükenmez I, Ersen MS, Bakioğlu AT, Bícer A, Pamuk V. Dose dependent oxidation kinetics of lipids in fish during irradiation processing. Radiat Phys Chem 1997;50(4):407-14.
35. Gousseff M, Lanternier F, Ferroni A, Chandesris O, Mahlaoui N, Hermine O, *et al.* Enterobacter cloacae pyomyositis complicating chronic granulomatous disease and review of gram-negative bacilli pyomyositis. Eur J Clin Microbiol Infect Dis 2013;32(6):729-34.
36. Steger PJK, Mühlebach SF. Lipid peroxidation of i. v. lipid emulsions in TPN bags: the influence of tocopherols. Nutr 1998;14(2):179-85.