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Stability of TPN mixtures compounded from Lipofundin S and Aminoplex amino-acid solutions: Comparison of laser diffraction and Coulter counter droplet size analysis

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Summary

We have studied the stability of a range of total parenteral nutrition (TPN) mixtures compounded from Lipofundin S and Aminoplex amino acid solutions. Droplet size was studied for 180 days using both light scattering and electrical zone sensing techniques; additionally, the chemical stability was monitored via the pH and osmolality of the mixtures. All the mixtures were stable for 90 days, but some irreversible flocculation was observed in all after 180 days. This appeared to be due to the prolonged storage of the bags in one position, and suggested that TPN mixture stability could be enhanced by occasional remixing of creamed droplets. The Coulter counter was able to detect the gradual formation of oil droplets larger than 1 μm in diameter, while the laser diffraction instrument was less sensitive to these droplets until significant coalescence had occurred by day 180. The results demonstrate the value of single particle zone sensing techniques for the study of TPN mixture stability.

Introduction

Parenteral fat emulsions are potentially highly variable products, since their raw materials (soya oil and lecithin) are of biological origin, and undergo considerable purification prior to use. The precise technology used to produce the emulsions also differs between manufacturers, and so it is not surprising that fat emulsions from different sources display different physical characteristics, such as varying droplet diameter and polydispersity. Given the potential for variation, it is remarkable that the differences observed in clinical use are so small.

The interproduct variation suggests that there may also be differences between the stability of total parenteral nutrition (TPN) mixtures made with emulsions from different manufacturers. Consequently, the introduction of a new product requires that not only its physical stability be evaluated, but also that of TPN mixtures compounded from it. We have performed a limited

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study of a range of mixtures using the emulsion Lipofundin S 20% (Braun). The regimens covered a range of therapeutic requirements, including low volume, high nitrogen, high electrolyte, and low osmolality.

This investigation also provided the opportunity to compare the sensitivity of different particle sizing technologies to the study of TPN mixture stability. This is potentially a difficult problem, since it is necessary to detect a small number of droplets larger than 1 μm in the presence of many more submicron drops (Washington, 1990b). The Coulter counter has been widely used for measurements in this size range, but many workers are now favouring laser diffraction instruments due to their ease of use.

Materials and Methods

The mixtures chosen for the study were selected from the mixtures available from Cheltenham Nutrition Services. These were C1 (standard requirements), C2 (high electrolyte), C3 (high nitrogen), C4 (low volume) and C5 (low osmolality).

TPN bags were compounded using the regimens listed in Table 1. Three bags of each mixture were produced to assess reproducibility; the bags were mixed at Cheltenham Nutrition Services using isolator technology. Lipofundin S 20% (batch 034581C) was kindly provided by Braun Medical Ltd, Aylesbury; Aminoplex 12 (batch 038011), Aminoplex 24 (batch 038025), Glucoplex 1000 (batch 021009) and Glucoplex 1600 (batch 018012) were kindly provided by Geistlich Pharmaceuticals, Chester. Glucose, electrolyte solutions, and Addamel were drawn from regular compounding unit stock. Ultrastab 3L EVA bags (Miramed) were used for all mixtures.

TABLE 1

Composition of regimens studied (all volumes in ml)

	C1	C2	C3	C4	C5
Aminoplex 12			500		1000
Aminoplex 24	500	500	500	500	
Lipofundin S 20%	500	500	500	500	500
Glucoplex 1000	1000				
Glucoplex 1600		1000	1000		500
Glucose 5%					1000
Glucose 50%				500	
Potassium chloride 15%		37.5		10	
Potassium phosphate 17%	20	20	20	20	10
Sodium chloride 30%		27		15	
Addamel	10	10	10	10	10

The bags were transported to Nottingham while cool and stored horizontally at 4°C. Samples (20 ml) were withdrawn through an 18 g needle for study after thorough mixing on days 1, 7, 14, 30, 60, 90 and 180. The following parameters were measured:

(1) Mean droplet diameter of droplets > 1.2 μm diameter using a Malvern Mastersizer with 300 mm lens. The size channel in which the largest droplet was detectable was also noted.

(2) Droplet size distribution above 1.2 μm using a Coulter TAII with 100 μm aperture. A 2 ml sample of emulsion was diluted to 200 ml in filtered water and 0.2 ml of this sample was added to 150 ml of electrolyte in the counting vessel. A 2 ml sample was drawn through the pinhole for counting. This dilution proved too small for some of the later measurements in which significant coalescence had occurred, and so further dilutions were made as required.

(3) The pH of the mixtures was measured using a Corning model 7 pH meter and combination electrode calibrated at pH 7 and 20°C. Mixtures were allowed to warm to room temperature before measurement.

(4) Osmotic pressure was measured with a Gonotec cryoscopic osmometer, using 50-μl samples. The instrument was calibrated with 0.3 Osm/kg potassium chloride.

Results

All Coulter counter size distributions decayed to background below 5 μm, and so the results are presented as a single sum of the counts between 1.2 and 5 μm present in 35 μl of original TPN mixture. This total count is shown in Fig. 1 for each mixture over 90 days. After 180 days, several of the mixtures showed so much aggregation that

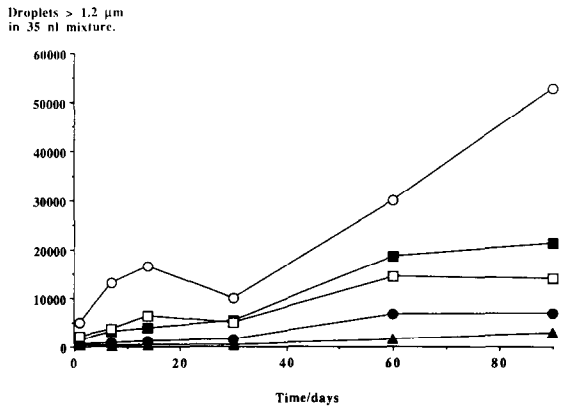

Fig. 1. Total Coulter counter droplet count between 1.2 and 5 μm for all mixtures over 90 days. (■) C1, (□) C2, (●) C3, (○) C4, (▲) C5.

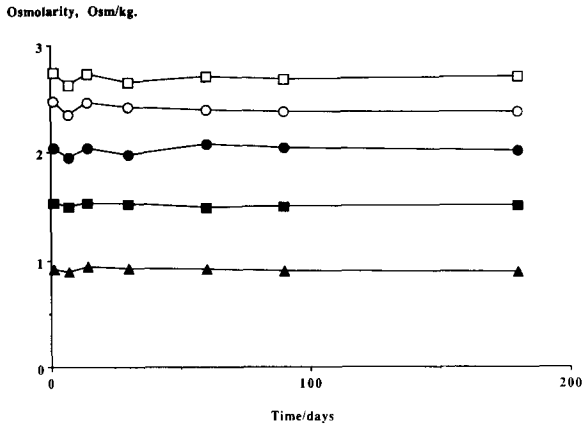

Fig. 3. Mixture osmolality over 180 days. (■) C1, (□) C2, (●) C3, (○) C4, (▲) C5.

it was not meaningful to measure them using the Coulter counter. All the mixtures demonstrated a general increase in droplet count with time, with the exception of the data obtained on day 30, which is slightly depressed.

The corresponding laser diffraction results are depicted in Fig. 2. These are the $Dv_{0.9}$ values (i.e., 90% of the volume of droplets detectable by the instrument are smaller than this value). Since the Mastersizer with this lens only detects droplets larger than 1.2 μm, this is not a true value for the total distribution, but only reflects the distribution of the largest droplets.

No significant changes were seen in the pH or osmolality (Fig. 3) of the mixtures over the study period. All the mixtures had a pH from 6.6 to 6.8, with the exception of the low volume (C4) mixture, which had a pH of 7.0–7.1.

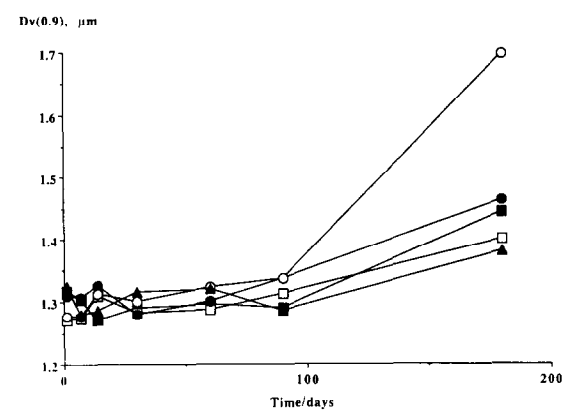

Fig. 2. Mastersizer $Dv_{0.9}$ values for all mixtures over 180 days. (■) C1, (□) C2, (●) C3, (○) C4, (▲) C5.

Discussion

The mixtures showed a considerable variation in stability, the most stable being those in which the emulsion was the most dilute (C5) or contained additional amino acids (C3). The least stable mixtures were the standard and low volume mixtures. Each bag of a particular mixture exhibited similar behaviour, suggesting that TPN mixture stability is deterministic. There has recently been much informal discussion among pharmacists concerning the variation in stability of TPN mixtures compounded in a similar manner from similar constituents, and it is encouraging that at least on the small scale of this trial, no such random variations were observed. We have previously suggested (Washington, 1990b) that part of the observed variability in TPN mixture

stability may be due to variations in fill volume of the components. Ongoing discussions with our colleagues in hospital pharmacies suggest that a further factor may be variation in compounding practises, both between centres and on a bag-to-bag basis.

The relative mixture stabilities can be understood in terms of their composition in a qualitative sense. The major destabilising factors in TPN mixtures are the ionic strength and presence of specifically binding electrolytes, which control the nonspecific and specific adsorption processes on the droplet surface, respectively (Washington, 1990a). All the mixtures contain the same amount of divalent specifically binding ions (from the Addamel component) but have varying ionic strengths. It is difficult to calculate this parameter, since ionization of the amino acids also makes a contribution to it, and we have recently shown (Washington et al., 1991) that amino acids bind electrolytes in TPN mixtures, thus further complicating the calculation of the exact ionic composition of the medium.

Consequently, we have chosen to use the osmolality as an approximate indicator of ionic activity. This is not a particularly rigorous approach but we are only seeking a qualitative comparison of the mixtures. Fig. 4 illustrates the stability of the mixtures, as represented by the Coulter counter data at day 90, plotted against the mixture osmolality. The mixture with the lowest osmolality (C5) is more stable than the standard regimen (C1). Increasing the osmolality would be expected to decrease the stability (i.e., increase the droplet count); this is observed for the low volume (C4) mixture. Mixtures C2 and C3 appear not to fit this pattern; C3 is anomalously stable, since it is a high nitrogen mixture, containing 50% more amino acid than the other mixtures. The stabilizing effects of amino acids are well-known (Washington et al., 1991). The high electrolyte mixture (C2) is also anomalously stable. This is not surprising since such a mixture is almost certainly charge reversed, and gains stability by virtue of having positively charged droplets.

We have suggested (Washington, 1990b) that the apparent stability of TPN mixtures may be modified by continuous remixing at the sampling

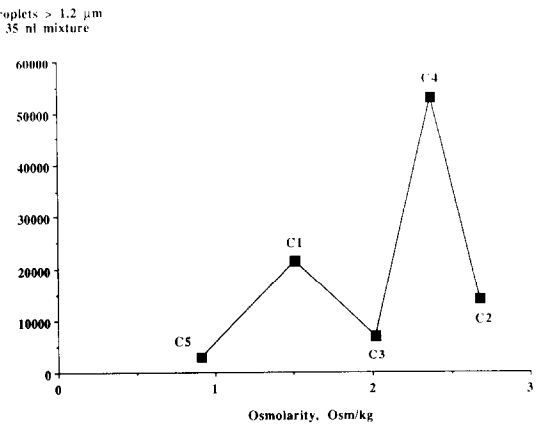

Fig. 4. 90 day Coulter counter data vs mixture osmolality.

times, and that a continually remixed bag may display improved stability with respect to a bag which is not agitated. Connected to this is the possibility that the orientation of the bag during storage may influence its stability. The present study does indeed suggest that this area should be explored further. In the first 90 days of the study, the bags were agitated monthly, and although slight creaming was visible, the bags were visually acceptable for clinical use. However, after 90 days, the bags were undisturbed until the 180 day sample was withdrawn. After this long undisturbed period, a significant amount of aggregated material was visible in many of the bags. This material had a lumpy or stringy appearance, and was not redispersible by agitation in the manner that is usually observed for a reversibly creamed mixture. The exact nature of this material is unclear, but it is possible that it may be made of flocs bound together by a film of free oil.

This phenomenon must be further investigated if we are to understand the nature of instability in TPN mixtures. Previous experiments have suggested that mixtures may have a finite lifetime, which is terminated by the rapid breakdown of the mixture in a catastrophic manner. This possibility may be an artefact arising from the study of particle size by laser diffraction methods. The droplet diameter as measured by laser diffraction (Fig. 2) shows no change until a particular time is

reached; this suggests that sudden coalescence occurs, possibly as a result of nucleation by large droplets. However, the Coulter counter data indicate a gradual and continuous coalescence leading to large droplets. This suggests that, in order to study the changes in size distribution and their influence on mixture stability, it may be necessary to examine mixtures which are continuously agitated. This may even apply to mixtures which display no major instability, since even the unflocculated emulsion droplets show a tendency to cream over the long experimental durations examined here.

A consequence of this possibility is that the stability of a mixture must be tested under the same conditions of storage that would normally be used in clinical practise, and that the stability of a mixture in the hospital may be poorer than that suggested by the manufacturer's data, which has been obtained on repeatedly sampled bags.

The most significant problem in the study of TPN mixtures is the establishment of acceptable limits of stability for clinical purposes. There are at present no useful data to allow stability limits to be evaluated. All the mixtures examined in this study were acceptable up to 90 days using the criterion that only small amounts of redispersible cream were present.

An alternative criterion based on the count of 5 μm droplets by Coulter counter is possible; however, the present study demonstrated that these droplets were so infrequent as to pose major sampling and statistical difficulties due to the small volume of the mixture examined. For example, the present counting protocol measures the droplet count in 20–30 nl of the mixture, and with care, the background count in this volume at 5 μm can be reduced to a few counts. An error of 1 count in 20 nl of mixture corresponds to 10^8 drops in a 2 l bag, which represents a detection limit of 6 mg of oil. Whether this represents a clinical hazard is not at present known.

Conclusions

The mixtures studied using Lipofundin S and Geistlich amino acids showed good stability for times up to 90 days. Breakdown after this duration appeared to be due to aggregation within a creamed layer, and it is possible that mixture stability could be enhanced by occasional agitation of the mixtures. The Coulter counter demonstrated a significantly greater sensitivity for the detection of large oil droplets in these systems than the Mastersizer.

Acknowledgements

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