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Ageing effects in parenteral fat emulsions: the role of fatty acids

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Summary

The effect of age on the pH, ζ-potential, and flocculation behaviour of a commercial fat emulsion of soybean oil stabilized by egg lecithin ('Intralipid 10%') has been studied. This has been compared to the behaviour of 'Intralipid 10%' to which oleic acid had been deliberately added. In both cases the critical flocculation concentration to calcium ions was increased, the ζ-potential became more negative, and the pH fell. The stability to electrolytes was in accord with the predictions of simple DLVO theory (Verwey and Overbeek, 1948), and the results suggested that the production of free fatty acids may account for the changes in the properties of fat emulsions with age. The implications for the formulation of total parenteral nutrition mixtures are discussed.

Introduction

Fat emulsions such as 'Intralipid' are widely used for parenteral nutrition in cases where essential fatty acid deficiency is likely to be a problem. They are used both alone and with admixtures of essential compounds such as amino acids, vitamins, and trace elements, as total parenteral nutrition (TPN) mixtures (Allwood, 1984). Although such emulsions are sufficiently stable for clinical use, two particular problems are associated with their stability.

Firstly, the shelf life of the unmixed emulsions is limited to 18 months at 4–8 °C. As the emulsion ages its pH falls; this has been attributed to the production of free fatty acids (Boberg and Hakansson, 1964; Kawilarang et al., 1980). Secondly, the emulsions are rapidly flocculated by electrolytes (Dawes and Groves, 1978; Whateley et al., 1984); this causes difficulties in the formulation of total parenteral nutrition mixtures in a single bag, although a number of groups have made advances in this direction. (Black and Popovich, 1981; Burnham et al., 1983)

In this study we have examined the flocculation of various batches of 'Intralipid 10%' by calcium ions, and the changes in pH and ζ-potential that occur with age. These results have been compared to the behaviour of 'Intralipid 10%' to which oleic acid, a typical product of phospholipid and oil oxidation, has been added, to ascertain to what extent the changes in behaviour with age can be ascribed to fatty acid production.

Materials and Methods

The flocculation of emulsions by electrolytes was assessed by measuring the change in ab-

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sorbance with time after the addition of calcium chloride solution. This was performed using an absorptiometer constructed by the authors and interfaced to a BBC microcomputer for data analysis (to be described elsewhere). Absorbance was measured at 450 nm. The emulsions were diluted to an oil phase volume of 0.1% in pH 7 HEPES buffer (Sigma) before being mixed with an equal volume of calcium chloride solution and measuring the rate of change of absorbance. In all systems examined the absorbance increased linearly with time for the 1–2 min duration of the measurement. Flocculation rates are quoted as rate of change of absorbance, A.U. s^{-1}.

ζ-Potentials were measured in pH 7 HEPES buffer adjusted to an ionic strength of 0.01 M with sodium chloride. Measurements were made by quasi-elastic laser light scattering (Zetasizer II, Malvern Instruments).

The pH of the emulsions was measured with a Corning model 7 pH meter and electrode, and was accurate to ± 0.1 pH unit.

'Intralipid 10%' (KabiVitrum, Stockholm) was obtained from the local hospital pharmacy at various times as bottles from newly received batches which were subsequently stored at 4° C as recommended by the manufacturers. Only expired batches were examined in order to study the ageing process; the newest batch studied here (52311) behaved identically to a sample which had not expired. Intralipid containing oleic acid (BDH reagent grade) was prepared by adding 2% oleic acid to 'Intralipid 10%' (Batch 52311, expiry date 1.5.86), mixing with a blender (Silverson Machines small laboratory emulsifier plus standard micro head), then sonicating for 15 min at 10° C (Dawe Soniprobe model 7532, 50 W power). This emulsion was diluted to the desired concentration of fatty acid with 'Intralipid 10%' from the same batch, and the mixtures were then sonicated again for 10 min to aid redistribution of the acid among the oil globules. Finally the mixtures were allowed to age for 48 h at 20° C prior to use.

Results

Fig. 1 shows the flocculation by calcium ions of batches of 'Intralipid 10%' of varying ages. The youngest emulsion (age 23 months, batch 52311)

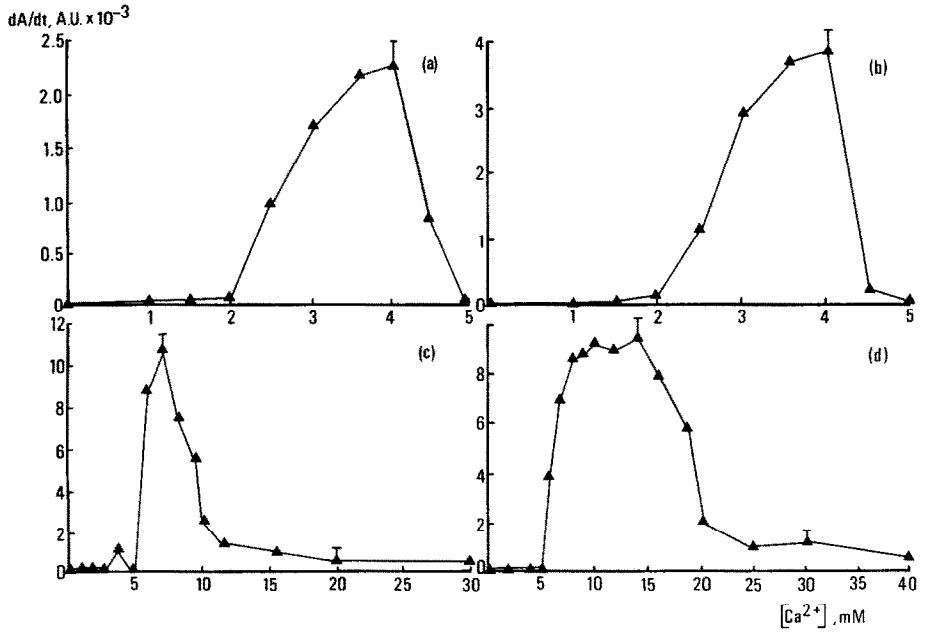

Fig. 1. Flocculation rate of 'Intralipid 10%' with added calcium ions. a: batch 52311, age 23 months. b: batch 1921466, age 38 months. c: batch 1910359, age 57 months. d: batch 1910228, age 59 months.

had a critical flocculation concentration (c.f.c) between 2 and 2.5 mM calcium, passed through a state of maximum flocculation at 4 mM calcium, and became stable again at 5 mM calcium. An emulsion sample of age 38 months (batch 1921466) showed similar behaviour. Older emulsions (batches 1910359 and 1910228 of ages 57 and 59 months respectively) showed a different behaviour. Flocculation commenced at 4–5 mM calcium, reached a broad maximum at 6–9 mM calcium, then slowly declined toward zero; however complete stability was not attained even at high (40 mM) concentrations of calcium. This contrasts with the behaviour of younger emulsions which were stable above 6 mM calcium. In addition the peak flocculation rates of the older emulsions were 3–4 times larger than those observed with the newer batches.

The flocculation of 'Intralipid 10%' containing 0.1% w/v oleic acid is shown in Fig. 2. Flocculation commenced at 4 mM of calcium, peaked at 7 mM, then declined toward zero. Again, complete restabilization was not observed at the highest calcium concentrations studied, and the peak flocculation rate was 3 times that of the 'Intralipid 10%' to which no oleic acid was added.

The ζ-potential of 'Intralipid 10%' as a function of age is shown in Fig. 3. Fresh emulsion had a ζ-potential of -47 mV, which then became more negative in a nearly linear manner at a rate of ca. -0.36 mV/month. Fig. 4 shows the ζ-potential of 'Intralipid 10%' to which varying amounts of oleic acid were added. The ζ-potential

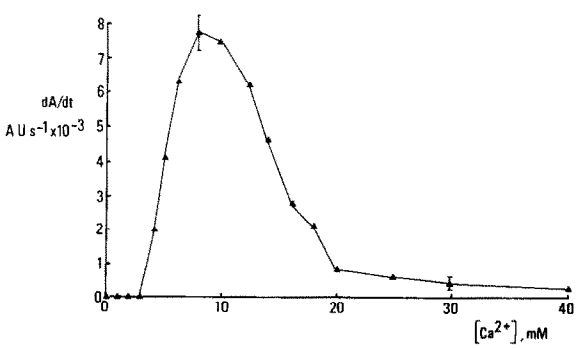

Fig. 2. Flocculation rate of 'Intralipid 10%' with 0.1% oleic acid added, as a function of added calcium ion concentration.

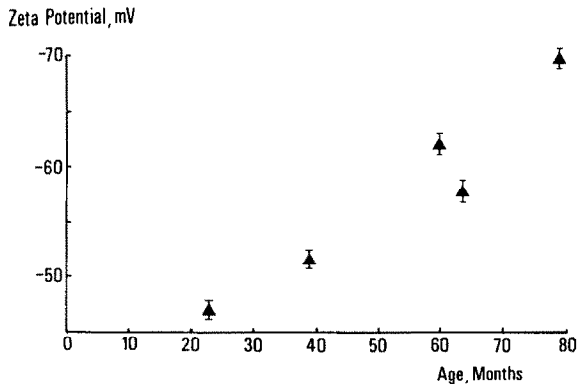

Fig. 3. ζ-Potential (at pH 7) of 'Intralipid 10%' as a function of age.

became more negative, rapidly at first, then linearly to -70 mV at 2% added oleic acid.

Fig. 5 shows the pH of 'Intralipid 10%' as a function of age. While newly prepared Intralipid is adjusted to pH 7 by the manufacturers, this reduced with age to 4.4. The pH varied widely from sample to sample, but the trend was clearly discernible.

Addition of oleic acid to 'Intralipid 10%' (Fig. 6) rapidly reduced the pH to 5.5 when measured 48 h after preparation. The samples creamed within 7 days of preparation, those containing the most fatty acid creaming first, and oil separation was visible in all samples after 28 days. The pH of all samples was then in the range 4.0–4.4.

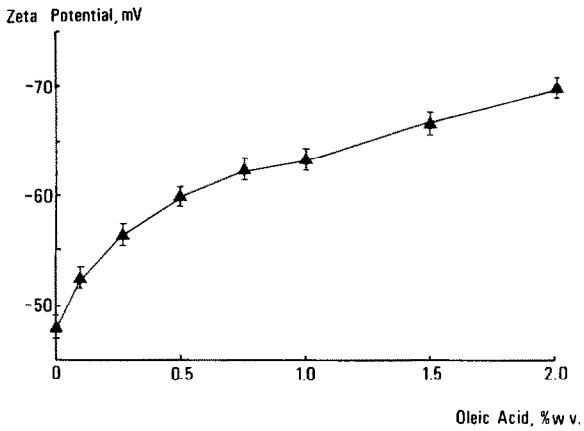

Fig. 4. ζ-Potential (at pH 7) of 'Intralipid 10%' as a function of added oleic acid.

calcium. Charge reversal in this manner implies that the calcium ion is specifically adsorbed to binding sites on the surface (Modi and Fuerstenau, 1957). We have previously suggested that this binding is to minor components in the interfacial layer, probably phosphatidic acid or phosphatidylserine (Burnham et al., 1983).

Older batches of 'Intralipid 10%' showed a critical flocculation concentration at higher calcium concentrations. This suggests that their initial ζ-potential was more negative than that of the fresh material, and Fig. 3 confirms this. Initially we can propose that some species, negatively charged at pH 7, was slowly produced in the interfacial layer during ageing, thereby causing the ζ-potential to become more negative. If we further assume, as Boberg (1964) and Kawilarang et al. (1980) have suggested, that the species was a fatty acid, then the decrease in pH can be ascribed to the hydrogen ions produced by its ionization. Interestingly, the results presented by Kawilarang et al. are not consistent with this hypothesis. They found that the ζ-potential of Intralipid became less negative with age. Their ζ-potential measurements disagree with those found here, and are also inconsistent with the proposed production of free fatty acids. These would be fully ionized at pH 7 and would cause the ζ-potential to become more negative with increasing age. However, Kawilarang et al. did not explicity state the pH or ionic strength at which the measurements were made.

It is also worthy of note that ageing increased the maximum flocculation rate of the emulsion by electrolyte, (i.e. the flocculation rate at the point of zero charge), and caused charge reversal to be a much more difficult process, requiring much higher concentrations of specifically adsorbed ions than for a fresh sample. Simple DLVO theory (Verwey and Overbeek, 1948) cannot account for these changes in behaviour, which may be due to interaction between the calcium binding sites and interfacial fatty acids.

The deliberate addition of oleic acid to 'Intralipid 10%' caused changes in flocculation behaviour, pH and ζ-potential which are qualitatively similar to those produced by ageing. Thus, the ζ-potential became more negative due to the accumulation of negatively charged ionized carboxyl groups on the surface, and this caused an increase in the c.f.c. caused by calcium ions. An increase in peak flocculation rate and increased resistance to charge reversal were also evident. Thus it is reasonable to conclude that the observed changes in the flocculation behaviour of 'Intralipid 10%' with age were due to the production of free fatty acids. Exposure to air caused similar changes to the flocculation profile, and it would appear that this is a sensitive indicator of the condition of the emulsion and the adequacy of the storage regimen.

The present work suggests that older batches of 'Intralipid 10%' can support a greater load of added electrolyte than more recent batches. Burnham et al. (1983) have given a formula for the maximum amount of electrolyte that can be added to a total parenteral nutrition mixture, and it is reassuring that ageing processes increase this, providing a 'safety margin' for formulation. However, the amount of fatty acid present in the emulsion will be critically dependent on the storage history, and it would be unwise to exceed the suggested electrolyte concentrations unless additional stabilizing factors (such as the presence of amino-acids) are known to operate.

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