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The electrokinetic properties of phospholipid-stabilized fat emulsions

C. Washington, A. Chawla, N. Christy and S.S. Davis

Department of Pharmacy, University of Nottingham, Nottingham (U.K.)

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Summary

A study has been made of the electrokinetic behaviour of fat emulsions stabilized with model phospholipid mixtures, consisting of uncharged phosphatidylcholine doped with small quantities of phosphatidylserine, phosphatidylglycerol or phosphatidic acid. All the negatively charged lipids caused a similar negative zeta (ζ) potential to be developed on the emulsion droplets in the absence of divalent ions. Increasing amounts of negative phospholipid increased the droplet charge and caused the critical flocculation concentration and point of zero charge of the emulsion to be shifted to higher electrolyte concentrations. All the model systems had critical ζ potentials in the region -22 to -28 mV. Systems with a larger proportion of negatively charged lipids developed a lower positive charge in the charge-reversed region, leading to a lower stability to high concentrations of added electrolyte. The consequences of these studies for the stability of total parenteral nutrition mixtures is discussed. In particular the wisdom of attempting to maximize droplet charge in order to achieve long shelf-life is questioned when the emulsions are used in total parenteral nutrition (TPN) mixtures of high electrolyte content.

Introduction

Patients receiving intravenous nutrition have a need for essential fatty acids, which is usually provided by intravenous infusion of an emulsion of triglyceride oils in water, emulsified by lecithin. These systems, typified by 'Intralipid 20%' have a mean droplet diameter of 200–300 nm and a shelf-life of 1–2 years. They are charge-stabilized colloids, the droplets possessing a surface charge of -40 to -50 mV when freshly prepared, although this can increase with age due to the production of fatty acids by phospholipid hydrolysis (Davis et al., 1985; Washington and Davis, 1987).

Although these emulsions are highly stable, in clinical practice they are usually admixed with other materials, specifically electrolytes, carbohydrates, amino acids and trace elements (see e.g. Allwood, 1984). The resulting total parenteral nutrition (TPN) mixture has relatively poor stability since adsorption of electrolytes to the emulsion droplets causes a reduction in their surface charge and consequent increase in droplet coalescence rates. The situation is complicated by the effects of amino acids, which can reduce the destabilizing effect of the electrolytes; how this is achieved is not yet fully understood.

The charge on the droplet arises from the lecithin emulsifier. Lecithin is a mixture of phos-

Correspondence: C. Washington, Department of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

pholipids whose exact composition depends on the source, usually eggs or soya beans, and to a smaller extent the environmental factors at work during its biosynthesis in vivo. The major constituents are phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which are unionized at physiological pH, and do not contribute to the overall surface charge. Additionally phosphatidylserine (PS), phosphatidic acid (PA) and phosphatidylglycerol (PG) comprise 2–5% of the total lipid, and these are negatively charged at pH 7. These ionized minor components usually contribute a major proportion of the surface charge.

The electrophoretic properties of phospholipids themselves and their interactions with electrolytes are well understood from studies of liposomal model systems. Phosphatidylcholine binds divalent electrolytes weakly (Altenbach and Seelig, 1984; McLaughlin et al., 1978) but acidic phospholipids such as PS and PG bind these much more strongly (Papahadjopoulos, 1968; Van Dijck et al., 1978; Lau et al., 1981; Macdonald and Seelig, 1987). The binding of monovalent cations is much weaker than the binding of divalent cations (Eisenberg et al., 1979). The interaction with divalent cations is thought to proceed through the production of a 1 : 2 ion/lipid complex, a phenomenon which usually causes lateral phase separation of the different lipid constituents within the membrane (Findlay and Barton, 1978; Ganesan et al., 1982; Florine and Feigenson, 1987) and promotes fusion or aggregation in a liposomal suspension (Liao and Prestegard, 1979; Yoshimura and Aki, 1985; Bental et al., 1987).

The behaviour of phospholipids at the surface of fat emulsion droplets is similar, in that divalent ions such as calcium are strongly adsorbed whereas monovalent ions are more weakly adsorbed. Calcium is bound strongly to the lipid surface, which leads to the phenomenon of charge-reversal of the droplet, whereas sodium does not bind sufficiently strongly to demonstrate this effect. However, commercial fat emulsions and lecithins contain a considerable number of components, which makes the detailed study of such systems complex and difficult. In particular the calcium can be bound to mixtures of PS, PG and phosphatidic acid.

We have chosen to use simplified model mixtures of phospholipid emulsifiers to study the behaviour of the systems in more detail. In this way it has proven possible to study the contribution of each lecithin component to the overall behaviour of the fat emulsion. We have used pure dipalmitoylphosphatidylcholine as an emulsifier which is uncharged at pH 7 in the absence of divalent ions. To this, small quantities (0–10% w/w) of negatively charged phospholipids have been added. The ζ potential, calcium binding and electrolyte stability of emulsions stabilized with these phospholipid mixtures have been studied.

Materials and Methods

Soya oil was purchased locally from J. Sainsbury Co. and used without further treatment. All phospholipids were obtained from Sigma. Dipalmitoylphosphatidylcholine (DPPC, no. P-0763), phosphatidylserine (P-6641), phosphatidic acid (P-4393) and phosphatidylglycerol (P-0514) were used as received and stored at $-20\,^{\circ}C$. Phosphatidylserine was used immediately on arrival since it is unstable. HEPES buffer was obtained from Sigma; calcium chloride was BDH Analar grade. Chloroform was redistilled from May & Baker reagent grade. Water was freshly distilled since emulsions diluted into water which had been allowed to stand in a storage vessel showed anomalously high negative surface potentials. All glassware was rinsed thoroughly with distilled water before use to remove traces of ionic surfactants.

Preparation of phospholipid mixtures and emulsions

DPPC (120 mg) was weighed into a 25 ml RB flask and the appropriate quantity of PS, PG or PA was added as a dilute chloroform solution from a calibrated microsyringe. A further small amount of chloroform was added to dissolve the phospholipids and the solution evaporated under vacuum to ensure that the lipids were thoroughly mixed. The lipids were then dispersed in water (9 ml) by bath sonication. Soya oil (1 ml) was added and the whole emulsified while immersed in ice using an Ultrasonic probe (Dawe 7532B; 60 W, 15 min with 2×5 min cooling periods).

The proportion of negatively charged phospholipid is quoted as a fraction (w/w) of the total phospholipid present; thus e.g. '1% PG' means that the emulsifier contains 1% PG, not the emulsion.

ζ potential measurements

ζ potentials were measured by quasielastic laser light scattering using a Malvern Zetasizer II equipped with a 3 mm PC4 cell. Emulsions were diluted into 1 mM HEPES buffer of pH 7.4 containing the appropriate concentration of calcium chloride. It is important to keep all buffers at low ionic strength in order to measure the true ζ potential.

Electrolyte stability profiles

The flocculation rate of emulsions diluted into electrolyte solutions was determined by turbidimetry as described previously (Washington and Davis, 1987). Unless otherwise stated, flocculation rates were determined at pH 7, 20 °C and an oil phase volume of 0.05%.

Results

Fig. 1 shows the ζ potential of emulsion droplets stabilized by DPPC with varying quantities of PG at pH 7. The droplets had a low charge (−1 mV) when stabilized with pure DPPC but the presence of increasing quantities of PG charged the droplets to over −60 mV when the emulsifier contained 10% PG.

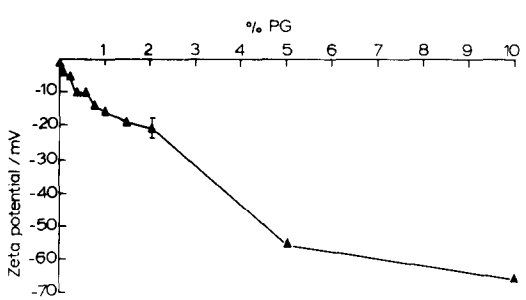

Fig. 1. ζ potentials of emulsions stabilized by 2% DPPC containing 0–10% phosphatidylglycerol.

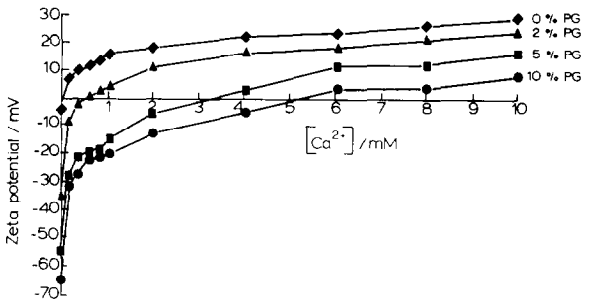

Fig. 2. ζ potentials of emulsions stabilized by 2% DPPC containing 0–10% phosphatidylglycerol in the presence of 0–10 mM calcium chloride.

Fig. 2 shows the ζ potential of droplets stabilized with DPPC/PG mixtures as a function of calcium concentration. The emulsion containing no PG had a small negative surface charge, but the adsorption of calcium caused a positive charge to be gradually developed to a maximum of +30 mV. Increasing the proportion of PG in the

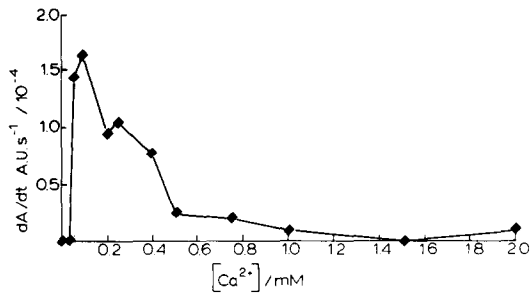

Fig. 3. Flocculation of emulsion stabilized by 2% DPPC by calcium chloride.

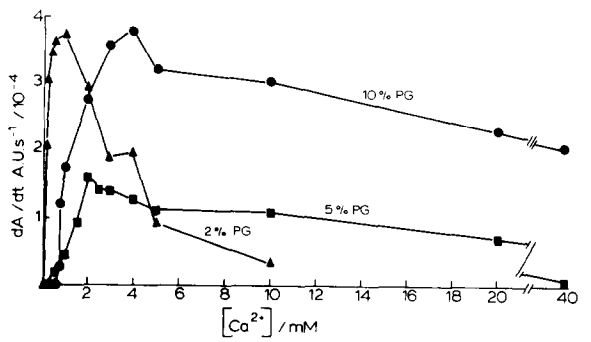

Fig. 4. Flocculation of emulsions stabilized by 2% DPPC containing 0–10% phosphatidylglycerol in the presence of 0–10 mM calcium chloride.

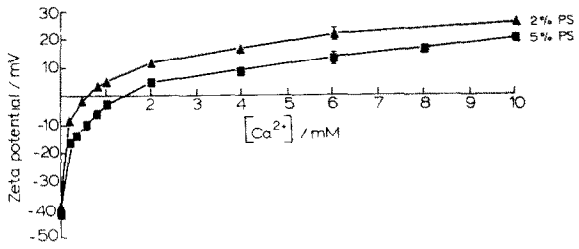

Fig. 5. ζ potentials of emulsions stabilized by 2% DPPC containing 2–5% phosphatidylserine in the presence of 0-10 mM calcium chloride.

emulsifier caused the droplet charge to become more negative in the absence of calcium, but as the calcium concentration was increased, the surface charges on all emulsions were first neutralized, then reversed to produce a positive potential. The calcium concentration at which the droplet charge was zero (the point of zero charge or PZC) increased with increasing PG concentrations from 0.1 mM for the pure DPPC stabilized emulsion, to 5.4 mM for the emulsion stabilized by 10% PG in DPPC.

The calcium flocculation profile of the emulsion stabilized by pure DPPC is shown in Fig. 3. Flocculation commenced at a calcium concentration of 0.05 mM and reached a maximum at 0.15 mM. The flocculation rate then rapidly decreased to below the detection limit at 1.5 mM calcium.

Increasing the proportion of PG in the emulsion (Fig. 4) had two effects on the flocculation profile. Firstly the calcium concentration producing the maximum flocculation rate was increased with increasing PG content, from 1 mM (2% PG) to 2.5 mM (5% PG) and 4 mM (10% PG). Secondly the rate at which the emulsion regained stability at higher calcium concentrations decreased markedly with increasing PG concentration. The emulsion containing 5% PG emulsifier

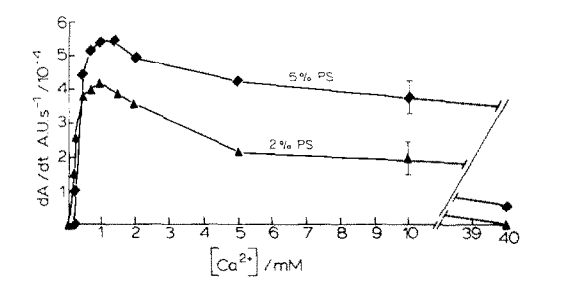

Fig. 6. Flocculation of emulsions stabilized by 2% DPPC containing 2–5% phosphatidylserine in the presence of 0–10 mM calcium chloride.

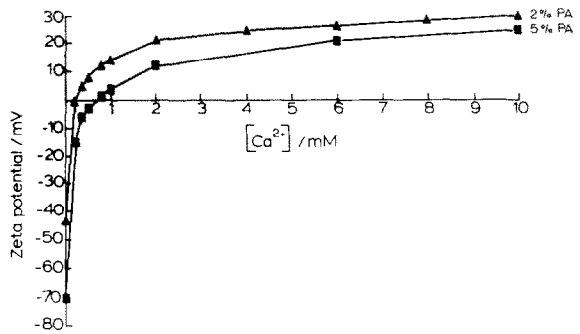

Fig. 7. ζ potentials of emulsions stabilized by 2% DPPC containing 2–5% phosphatidic acid in the presence of 0–10 mM calcium chloride.

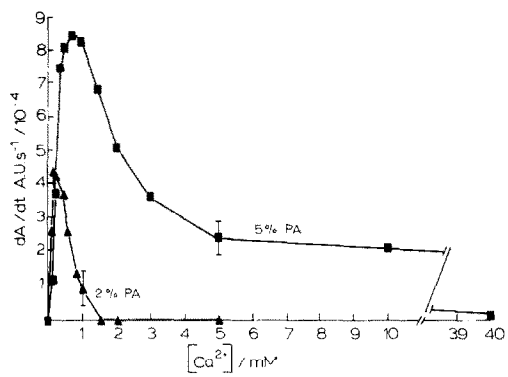

Fig. 8. Flocculation of emulsions stabilized by 2% DPPC containing 2–5% phosphatidic acid in the presence of 0–10 mM calcium chloride.

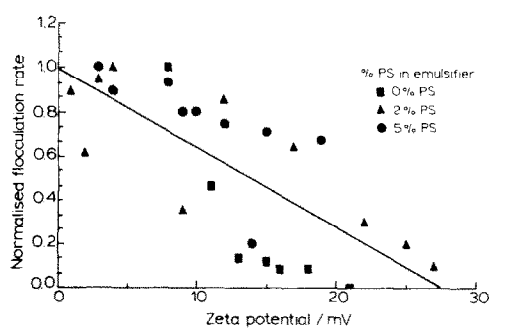

Fig. 9. Flocculation rate vs zeta potential of emulsions stabilized by DPPC/PS mixtures.

regained stability only at high (40 mM) calcium concentrations, whereas that containing 10% PG emulsifier was not restabilized even at this high calcium concentration.

Broadly similar results are obtained when phosphatidylglycerol was replaced by phosphatidylserine or phosphatidic acid. Phosphatidylserine (Figs. 5, 6) caused a negative surface charge which was gradually neutralized and reversed by calcium ions. 2% PS produced a PZC at 0.7 mM of calcium ion, while 5% PS produced a PZC at 1.5 mM calcium ion. The corresponding flocculation profiles show peak flocculation rates at 1 and 1.5 mM, respectively. Phosphatidic acid (2% and 5% in emulsifier) caused PZCs at 0.2 and 0.8 mM of calcium ion, with the corresponding peak flocculation rates also being seen at calcium concentrations of 0.2 and 0.8 mM (Figs. 7, 8).

Discussion

The relation between ζ potential and colloid electrolyte stability is well understood (see e.g. Hunter, 1981) and we have previously discussed this in relation to parenteral fat emulsions (Washington and Davis, 1987). The critical flocculation concentration occurs at a specific ζ potential; this can be found by plotting the flocculation rate (normalized to its peak value at the PZC) against the absolute value of the surface potential and extrapolating to zero flocculation rate. This analysis has the advantage that data in the charge-reversed domain can contribute to the estimate of the critical zeta potential. This has been performed in Fig. 9, which contains the data for all emulsions stabilized by PS/DPPC mixtures. The scatter is largely due to the difficulty in measuring accurately the flocculation rates, and to a first approximation the critical ζ potential obtained is constant, i.e. not dependent in any systematic manner on PS concentration. The same type of analysis is shown in Figs. 10 and 11 for PG- and PA-doped emulsions, respectively. The critical ζ potentials for PG-, PS- and PA-stabilized emulsions are 25 ± 2 mV, 27 ± 3 mV and 22 ± 2 mV, respectively, the differences between these values being insignificant. For comparison,

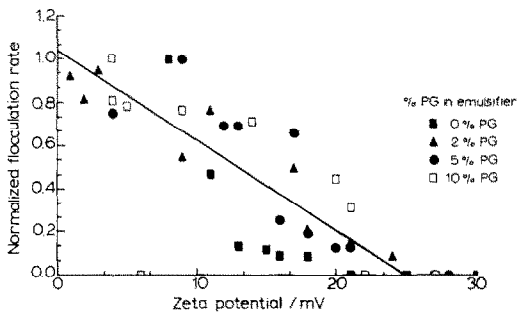

Fig. 10. Flocculation rate vs ζ potential of emulsions stabilized by DPPC/PG mixtures.

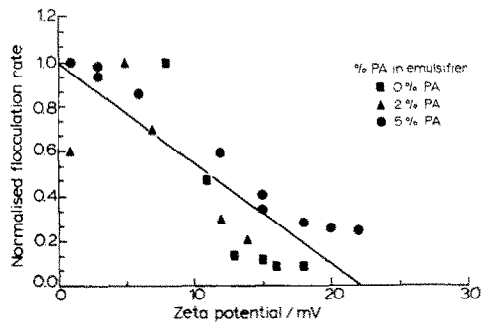

Fig. 11. Flocculation rate vs ζ potential of emulsions stabilized by DPPC/PA mixtures.

Fig. 12 shows the same analysis for 'Intralipid 10%' using data from Washington and Davis (1987). In this system the critical ζ potential is 11 ± 1 mV, significantly lower than that of the model systems studied here. This is despite the fact that the interfaces in both systems are composed of phospholipids and that the charge is due to ionization of acidic phospholipids. The difference could be due to two factors. Firstly it is

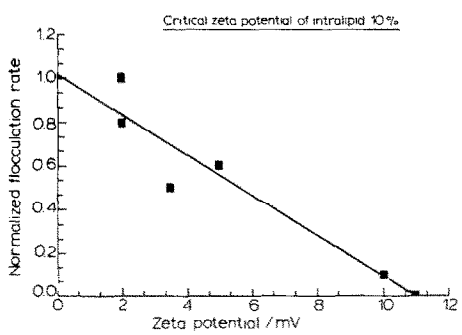

Fig. 12. Flocculation rate vs ζ potential of 'Intralipid 10%'.

possible that the impure lipids used to stabilize 'Intralipid 10%' contain some minor component of unforeseen importance. Secondly, the saturated phospholipid emulsifiers used in the model systems are all in the gel phase at room temperature, whereas Intralipid is stabilized by unsaturated liquid phase phospholipids. The difference in emulsifier film structure may be of importance.

Despite these differences in behaviour, the model systems studied here display many similarities to the commercial fat emulsion. Increasing quantities of negatively charged phospholipids make the droplet more negative in the absence of calcium, and the droplets bind calcium to charge-reverse the droplet. As the quantity of negatively charged phospholipid is increased, the critical flocculation concentration and point of zero charge occur at higher divalent ion concentrations. High concentrations of negatively charged lipids prevent the calcium-induced positive charge from exceeding the critical zeta potential for the system, which causes flocculation to occur even at high calcium concentrations. This is the origin of the long 'tails' to the flocculation profiles. Similar behaviour is seen in aging emulsions due to increases in free fatty acids (Washington and Davis, 1987); the flocculation profile is shifted to higher calcium concentrations and a long 'tail' develops in the charge-reversed region. Although we have not yet systematically measured ζ potentials in fatty acid-containing systems in the presence of calcium, the ζ potential in the absence of calcium becomes more negative with increasing fatty acid content. It is suggested that this causes the system to be unable to achieve the critical ζ potential when charge-reversed by divalent ions in a similar manner to the model systems studied here. Consequently such systems will not show optimum stability in the presence of high (5–10 mM) concentrations of divalent electrolytes.

It could be argued that the stability of a fat emulsion could be increased by adding a negatively charged lipid (or a fatty acid) to increase the ζ potential. The studies performed here demonstrate that although this may increase the stability of the unmixed emulsion, the flocculation in mixed electrolyte systems would be markedly different, particularly when further modified by amino acids. Consequently it would be necessary to completely reappraise the stability of the commonly used TPN regimens before such systems could be safely used in clinical practise. In particular, attempting to increase the shelf-life of emulsions by adding fatty acids may lead to emulsions which show less than optimal stability in high electrolyte TPN mixtures.

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