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THE EFFECT OF ELECTROLYTES ON PHOSPHOLIPID-STABILIZED SOYABEAN OIL EMULSIONS

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SUMMARY

The properties of the droplets of a commercial phospholipid-stabilized soyabean oil emulsion, Intralipid 10%, have been investigated by laser centrifugal photosedimentometer (LCP) and Zeta Meter. The surface charge is readily affected by added electrolytes and in the presence of physiological saline is reduced to zero. This may account for the anomalous sedimentation behaviour previously observed for Intralipid emulsions and confirmed here for sodium and calcium chlorides. The effects produced by cations may be related to their valency but anions are broadly similar to each other. Differences observed in behaviour may be due to different degrees of binding of the cations on the phospholipid interface.

INTRODUCTION

Following the ingestion of a fatty meal, the absorbed fats appear in the bloodstream as emulsified droplets, called chylomicra, which consist of a triglyceride core surrounded by an even layer of phospholipid (Fraser, 1970; Frederickson et al., 1967). The chylomicra vary in size according to the species of animal involved in the studies and the diet but vary from around 25 nm to 3 μm in diameter (Schoefl, 1968; Hallberg and Wesall, 1964; Bierman et al., 1966). Schoefl (1968) was able to demonstrate by electron microscopy that the main feature of the interface was the amphiphilic phospholipid occurring as a monolayer although multilayers could also occur as surface lamellae.

In a conscious attempt to mimic chylomicra various phospholipid-stabilized oil systems have been proposed and developed for intravenous nutrition, since this type of product has the advantage that a relatively high energy source can be administered in a small volume of liquid. The best known of these parenteral emulsions is Intralipid (Vitrum AB, Stockholm) which consists of a fractionated soyabean oil stabilized with a fractionated egg lecithin and glycerol to ensure isotonicity. Recent investigations in this laboratory

have demonstrated that the size distribution of the material is similar to that reported for chylomicra (Groves et al., 1975; Jeppsson et al., 1976). The method of size analysis employed in these investigations was a laser centrifugal photosedimentometer (LCP). Under some conditions the centrifuged field was insufficient to allow the sedimentation of all the particles present in a sample of Intralipid, resulting in a stable dispersion which contains particles smaller than a critical or limiting diameter. This diameter may be estimated precisely and was shown to be affected by adding sodium chloride to the dispersion medium until, in the presence of sufficient electrolyte, the whole of the particulate material moved out of the analytical zone of the instrument. However, the effect of the electrolyte was anomalous in that over a range of concentrations which approximated to physiological saline, the limiting particle size became unexpectedly small, increasing slightly as the concentration was increased still further (Groves et al., 1975; Jeppsonn et al., 1976). There was no direct explanation of this effect although it appeared to be due to some influence of the electrolyte on the interfacial phospholipid.

MATERIALS AND METHODS

Materials

The Intralipid emulsions were supplied by Vitrum AB, Stockholm, and the formulations are given in Table 1. All other materials were of laboratory reagent grade, water being glass-distilled.

Instrumentation

Laser centrifugal photosedimentometer (LCP) – a prototype instrument as described by Groves et al. (1975) but modified in that a Spectra Physics Model 223 2 mW continuous helium-neon laser was used. Data was recorded using a Bryants Model 28000 chart recorder. Zeta Meter (Zeta Meter Inc. New York), operated as described in the instrument manual.

Methods

LCP. This investigation was a continuation of those described by Groves et al. (1975) and a similar method was employed. After gentle agitation samples (0.5 cm^3) of each emulsion were withdrawn aseptically from the rubber-capped container and diluted with 450 cm^3 of the appropriate vehicle. These were then mixed by gentle inversion of the

TABLE 1

FORMULATIONS OF THE INTRALIPID EMULSIONS INVESTIGATED

	a	b
Fractionated soyabean oil	10	20
Fractionated egg-lecithin	1.2	1.2
Dilute Glycerol (Ph. Int. 85%)	2.5	2.5
Diazepam	–	0.2
Water for Injection	to 100	to 100

container and introduced rapidly into the rotating disc of the centrifuge. The experiment was continued until the trace of optical density vs time came down to the original baseline (the disc filled with water only) or came to a point of stability, indicating that the system under investigation had a limiting particle size under these conditions. The limiting particle size experiments were run with the analytical sensor at a radius of 110 mm and the time at which the optical density became constant used to calculate the limiting size in accordance with Stokes Law.

$$d_{st_{lim}} = (18\,\eta \ln(R/S))/((\rho_1 - \rho_2)\omega^2_{t_{lim}})^{1/2}$$

where η = viscosity of the liquid continuum of density ρ_2;
R = analytical sensor radius and S the starting point, i.e. the radius of the centrifuge disc;
ρ_1 is the density of the particle of diameter $d_{st_{lim}}$ and
t_{lim} is the time taken for this particle to move from radius S to radius R at a rotational speed of ω radians sec^{-1}.

Densities and viscosities of the various electrolyte solutions were taken from tables or measured directly using standard methods. The density of the oil droplets was taken as 920 kg^{-3} (Merck Index 9th ed., 1976); no allowance was made for the lecithin interface, water of hydration or the presence of dissolved substance in the oil.

Particle size analyses could only be made when the optical density of the sedimenting system returned to baseline conditions. Measurements were then made at radii of 110 and 106 mm. Results were corrected by the method of Kamack (1951) and extrapolated to conditions of zero gravity (the centre of the disc) using the method described by Groves et al. (1975).

Zeta Meter. Each emulsion required to be diluted before examination and the 1 : 900 dilution used for the centrifuge experiments proved to be well suited to the Zeta Meter with regard to particle observation and counting. This also had the benefit that electrolyte conditions were similar in the two experiments.

A volume of 0.11 cm^3 of each emulsion was added to 100 cm^3 of the appropriate electrolyte solution in stoppered flasks and agitated to mix. The specific conductance of each solution was measured as a means of selecting the most suitable voltage to apply across the electrophoresis cell. Four replicates of each solution were examined and the transit times for a number of particles averaged for each of the replicates.

Initial experiments used electrolyte solutions at their natural pH. However, when examining the effect of pH on zeta potential, it was felt desirable to avoid the addition of unrelated ions in the form of buffer salts. For sodium chloride solutions, dispersions of Intralipid were prepared in separate solutions of sodium hydroxide and sodium chloride acidified to pH 2.0 whith hydrochloric acid. Molar strenghts were calculated with respect to the cation, and different mixtures were made in order to obtain a range of pH.

Polyvalent cations such as calcium and aluminium did not lend themselves to this treatment since their hydroxides were only sparingly soluble in water. For this reason only the effects of acidifying solutions of their chloride salts with hydrochloric acid were measured.

Zeta potential values were calculated from the observed electrophoretic mobilities

using the Helmholtz-Smoluchowski equation:

$$Zp = (EM.\ 4\pi\eta)/D$$

where η = viscosity;
EM = electrophoretic mobility;
Zp = zeta potential and
D = the dielectric constant of the suspending liquid at the temperature of measurement.

RESULTS

Particle size

The volume surface mean diameter for the emulsion containing diazepam (b, Table 1)

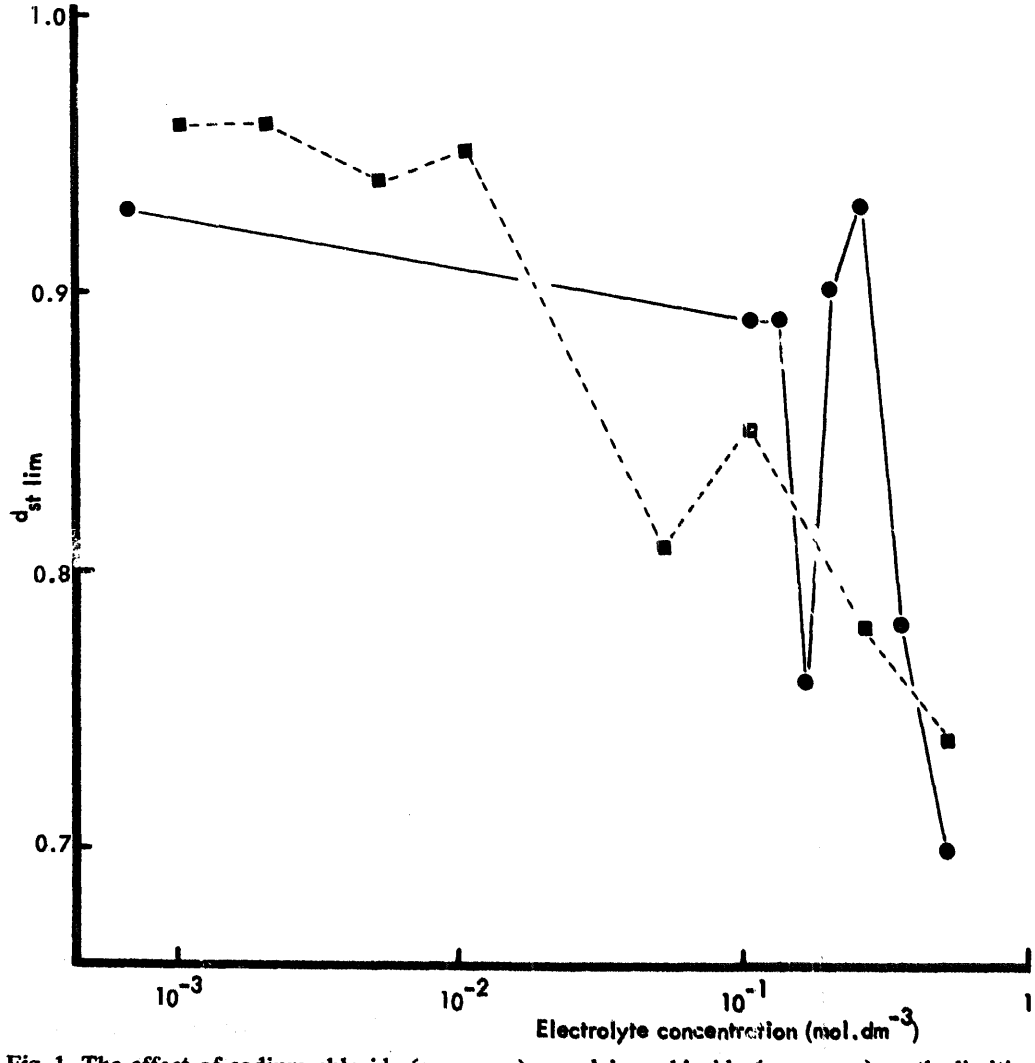

Fig. 1. The effect of sodium chloride (●———●) or calcium chloride (■- - - - -■) on the limiting particle diameter ($d_{st_{lim}}$) of Intralipid 10% measured by LCP (110 min radius and 3000 r.p.m.).

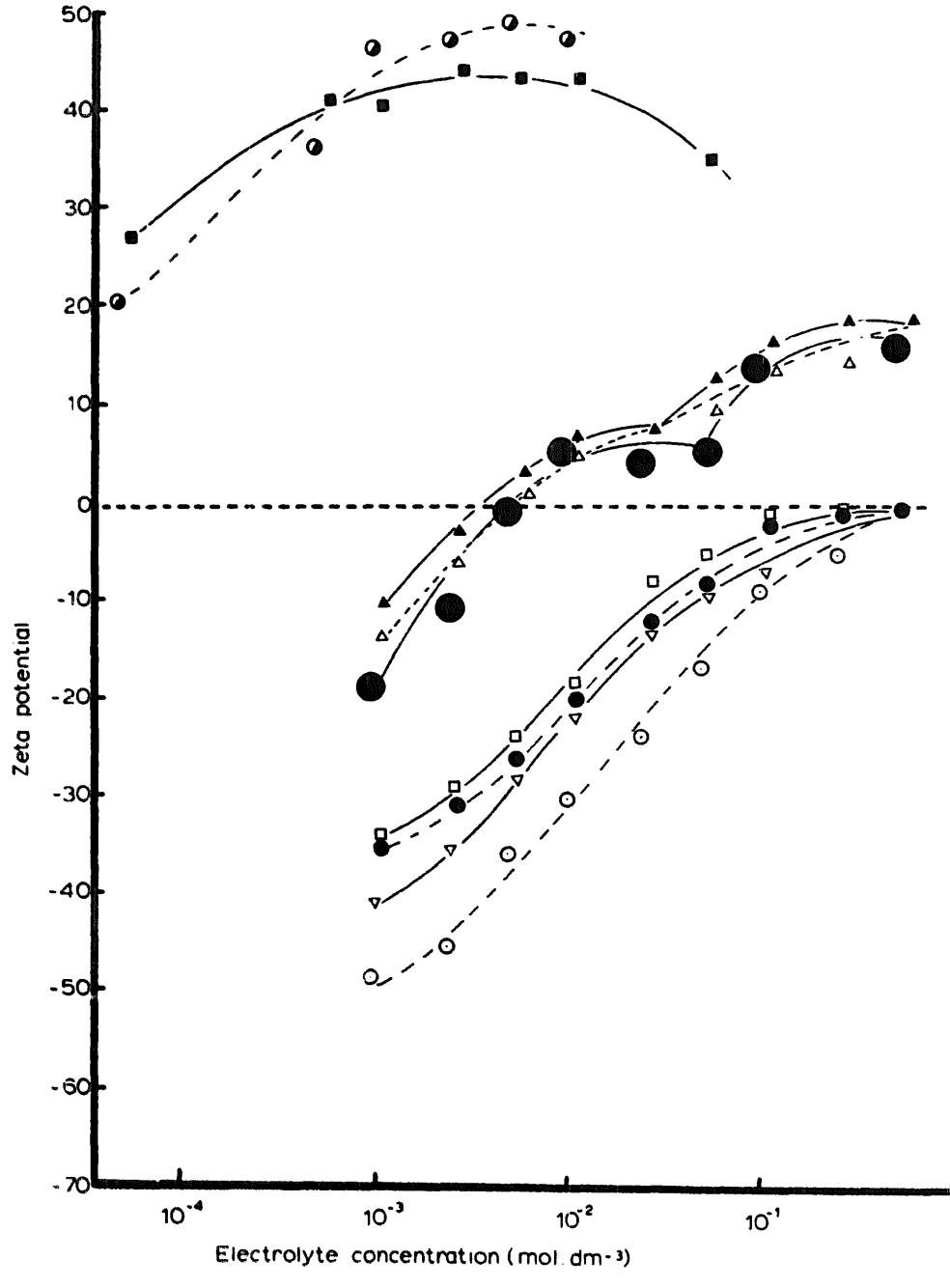

Fig. 2. The effect of electrolyte concentration and type on the zeta potential of:

Intralipid 10%	and	Intralipid + diazepam
NaCl	▽——▽	NaCl ⊙- - - - -⊙
KCl	●- - - - -●	$CaCl_2$ ●——●
LiCl	□——□	$AlCl_3$ ⊙- - - - -⊙
$CaCl_2$	▲——▲	
$MgCl_2$	△- - - - -△	
$AlCl_3$	■——■	

was 0.226 μm, close to that reported for a similar formulation (but containing acetylated monoglyceride as a stabilizer) by Jeppsson et al. (1976). However, no allowance was made for the interfacial phospholipid or any hydration effects which would change the density of the moving particles. Groves et al. (1975) showed that, by making a correction for a hydrated layer of suitable thickness and a density intermediate between that of the water and the soybean oil, results could be brought into line with those found by electron microscopy.

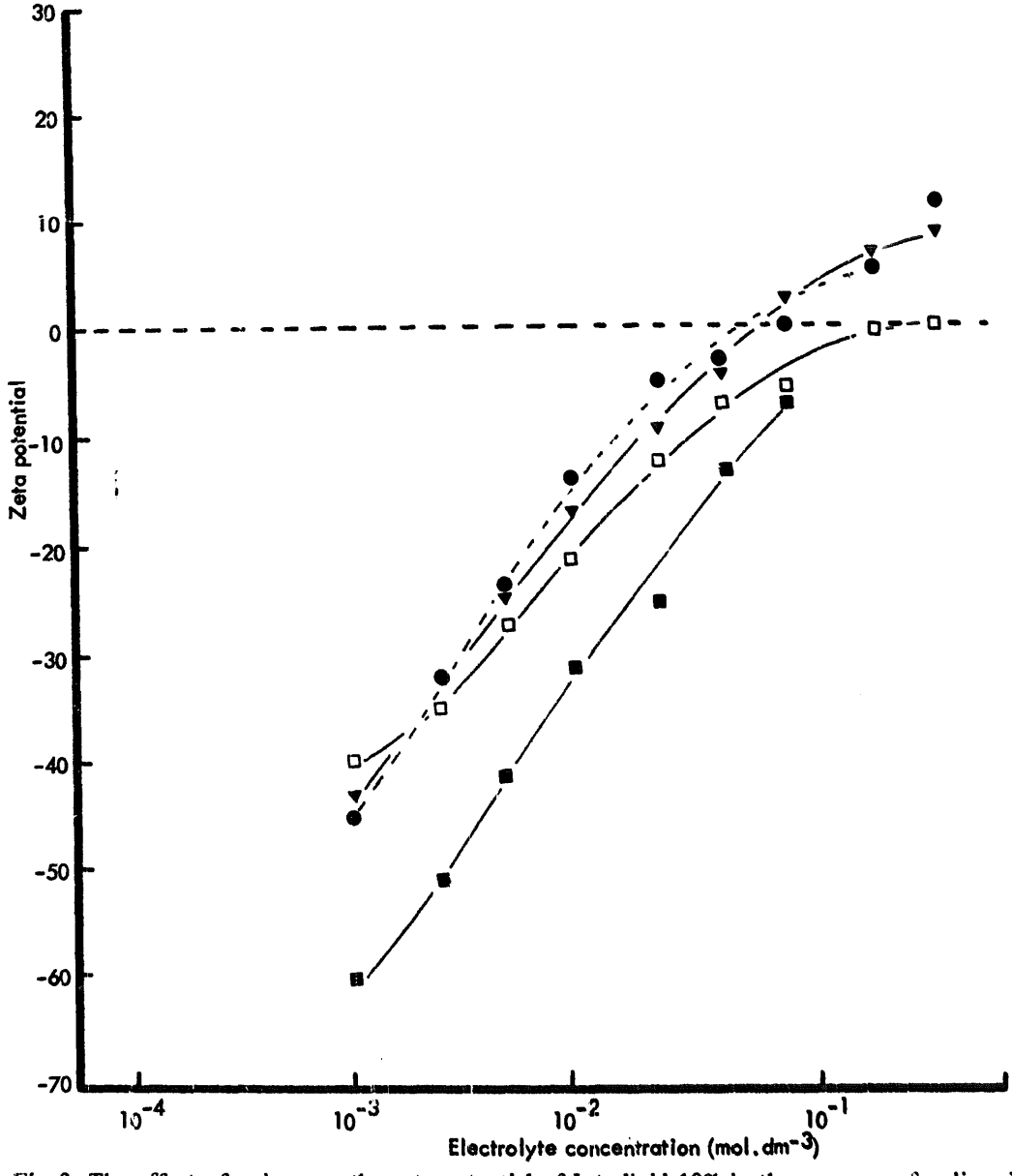

Fig. 3. The effect of anions on the zeta potential of Intralipid 10% in the presence of sodium ions. Sodium chloride, □——□; sodium citrate, ▼——▼; sodium pyrophosphate, ■——■; sodium sulphate, ●- - - - -●.

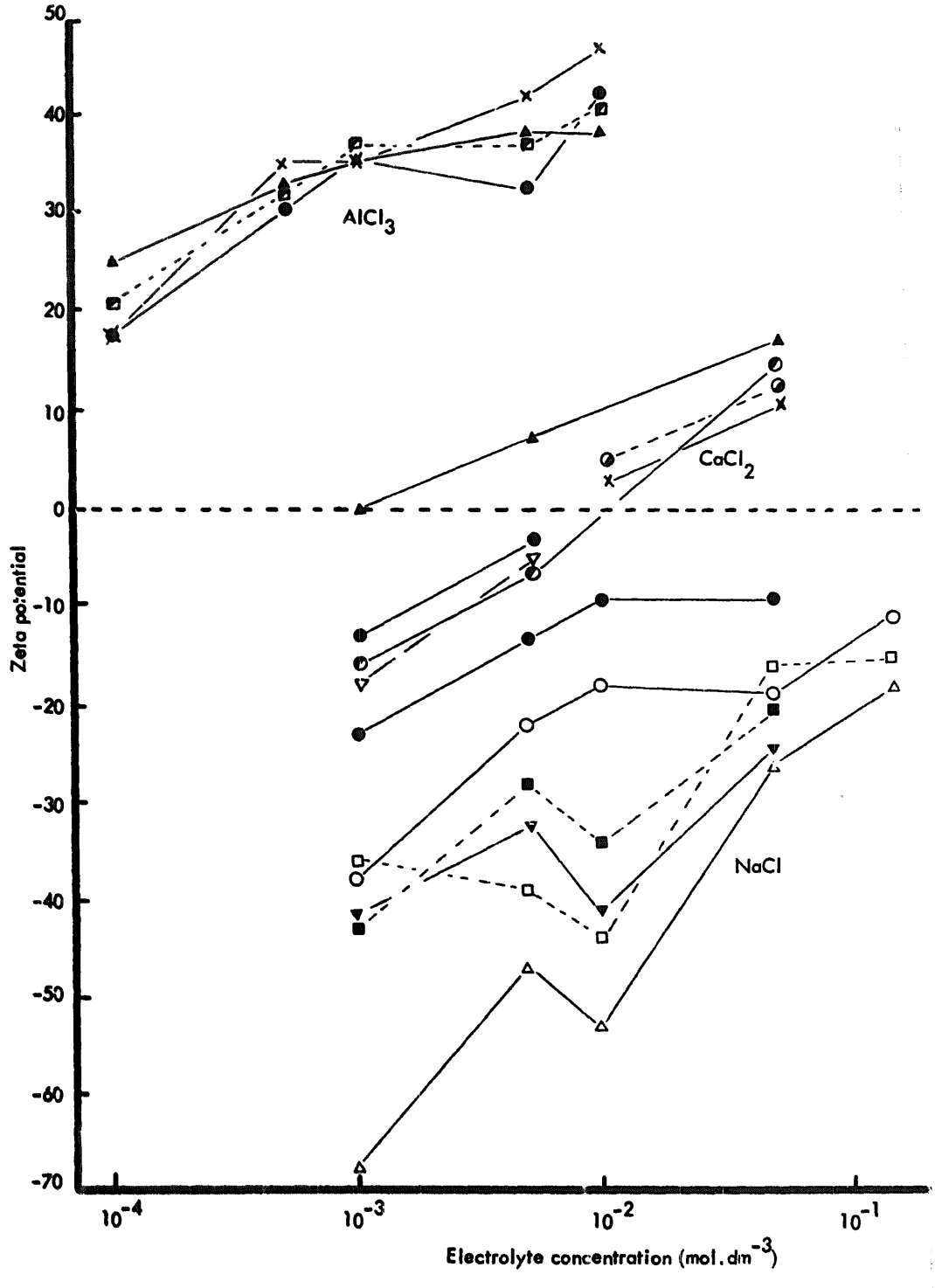

Fig. 4. The effect of pH and electrolyte concentration on zeta potential of Intralipid 10% in the presence of sodium chloride, calcium chloride or aluminium chloride. pH 1.5, ▲——▲; 3.0, ■- - - -■; 3.5, ●——●; 4.0, X——X; 4.5, ▽- - - -▽; 5.0, ○——○; 5.5, ◐——◐; 6.0, ◐- - - -◐; 6.5, ■- - - -■; 8.0, □- - - -□; 9.5, △——△; 11.0, ▼——▼;

Assuming a similar layer to be present, the mean Stokes' diameter was 0.36 μm, with a corresponding d_{vs} of 0.42 μm.

Limiting particle size

The limiting particle diameters of these samples of Intralipid are shown in Fig. 1 for sodium chloride and calcium chloride solutions and show the inflection observed previously for sodium chloride (Groves et al., 1975).

Zeta potential

Zeta potential measurements are summarized in Figs. 2–4.

DISCUSSION

A dispersion of Intralipid 10% particles in distilled water was found to have a zeta potential of 30 mV, ideal, according to Powis (1914), for ensuring emulsion stability. Even small amounts of electrolyte were able to bring about a change in this stabilizing charge. Monovalent cations (Na^+, K^+ and Li^+) all produced similar effects (Fig. 2). This observation agrees with those of Anderson and Pethica (1956) who showed that cations were not bound to lecithin monolayers except at relatively high concentrations (0.1–1 M). There was also little change in the surface potential below 0.1 M. The fact that the surface charge on the droplets is effectively zero at a sodium chloride level of 0.1–0.2 M is likely to be due to a change in the ionic nature of the double layer rather than the particle surface itself. This may be due to binding taking place after an ionic equilibrium has been set up in the double layer. Owing to the high specific conductivity of the saline solutions the possibility of charge reversal at even higher concentrations could not be investigated.

The similarity of the effect produced by calcium and magnesium ions is shown in Fig. 2. In these cases reversal of charge did occur, with a zero zeta potential being found at a much lower concentration than that measured for monovalent cations. An inflection occurred at around 0.05 M, approximately half that at which sodium ions are bound and this may be significant.

The Intralipid 10% system is obviously extremely sensitive to the presence of aluminium ions. There was a very rapid reversal of zeta potential which diminished at higher concentrations. This is not readily explained but is similar to the effect observed by Anderson and Pethica (1956) in terms of the surface potential. The relationship between concentration of electrolytes and response was in accordance with anticipation when considering ranking of the cations in the Hofmeister Series, and is consistent with results reported by Riddick (1968) for hydrophobic colloidal dispersions.

The number of charges on the anion would appear to be less significant (Fig. 3) in that the resultant curves are very similar to one another. The initial increase in negative zeta potential at low concentrations would, however, appear to be governed by the charge of the anion and the effects seen here are again similar to those observed by Riddick (1968). In the case of sodium citrate and sodium sulphate there was a reversal of charge of the Intralipid particles at higher concentrations. These anions have a higher affinity for water than chloride ions and may produce a sufficient contraction in the double layer to induce sodium binding at a lower concentration.

The effect of changing pH is shown in Fig. 4 and demonstrates that, over the range of pH 4–9, the zeta potential remained substantially the same over similar sodium concentrations. This finding is in agreement with that of Shah and Schulman (1967) who found that the surface potential of lecithin was constant between pH 4 and 8. Below pH 4 the phosphate group of the lecithin molecule is neutralized and a reversal of the zeta potential becomes possible. A similar effect was observed with calcium chloride (Fig. 4) but the opposite effect occurred in the presence of aluminium chloride. Suppression of ionization of aluminium chloride at low pH may account for this phenomenon.

Davis (1976) has reported a pH of 6.7 as the iso-electric point of Intralipid particles in a solution of ionic strength 0.145. This observation has not been confirmed since, under comparable conditions, 0.15 M sodium chloride, the iso-electric point was found to be nearer pH 3.0. However, it would seem that the iso-electric point of phospholipid-stabilized emulsions must be determined under conditions of constant cation concentration over the range of pH investigated. This is because the cations can exert a greater influence on the zeta potential than a change in the pH.

The presence of dissolved diazepam in the Intralipid oil phase appeared to have little or no influence on the properties of the oil droplets. This suggests that the diazepam has no effect on the interface so that it is likely to be confined to the hydrophobic core of the droplets.

The anomalous effect produced during the progressive addition of sodium chloride on the limiting particle diameter (Groves et al., 1975) has been confirmed. The actual size found here was somewhat larger than that found earlier but this may well be due to differences in batch and the age of the batch of emulsion examined. The inflection point was the same, 0.15 M, and this coincides with the region in which the zeta potential of the droplets approaches a value of zero. The subsequent apparent expansion of the particles as the sodium chloride concentration is increased is probably due to the expansion of the phospholipid film as a direct result of sodium binding (Shah and Schulman, 1967). Whilst there is almost certainly a film of water bound to the surface of each particle, the structure and thickness of this layer will be affected by the presence and type of electrolyte. Thus, not only is the overall particle size affected but so too is the particle density (Groves et al., 1975).

Two inflections appear when the limiting diameter is measured in the presence of calcium chloride, the first of which also corresponds to the point at which the zeta potential is zero. The second is more pronounced and coincides with an inflection observed in the curve relating electrolyte concentration and zeta potential (Fig. 2). This concentration is approximately half that of the level at which sodium chloride produces a similar effect. It may, therefore, be due to binding of the calcium ions and expansion of the phospholipid film.

In the presence of aluminium ions there was complete coalescence of the emulsion droplets at the air-water interface.

In conclusion, it would appear that the phospholipid-stabilized soyabean emulsion Intralipid 10% is sensitive to the presence of electrolytes. The surface charge is reduced to zero in the presence of physiological saline, suggesting that the system is least stable under these conditions. The effects of cations are related to their valency. Anions produce effects broadly similar to each other. Differences observed may be due to effects

produced on the phospholipid interface which allow a greater degree of binding of the counter-ion.

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