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The electrokinetic properties of phospholipid stabilized fat emulsions VI. Zeta potentials of Intralipid 20% in TPN mixtures

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Summary

The present paper studies the electrokinetic properties of Intralipid 20% in complex media representative of those used in total parenteral nutrition (TPN) mixtures. These mixtures contain amino acids, glucose, and electrolytes at high concentration. Zeta potentials were measured by laser doppler light scattering (Malvern Zetasizer 4) and by electrosonic analysis (Matec ESA 8000). The mixtures studied had zeta potentials between +4 and −6 mV, depending on the mixture composition, and the previously studied processes of competitive specific and nonspecific adsorption of electrolytes on phospholipid surfaces were clearly apparent. As far as we are aware, these studies are the first systematic measurements of zeta potentials in full-strength TPN environments that have been reported.

Introduction

The electrokinetic properties of parenteral fat emulsions such as Intralipid have been widely studied in order to rationalize the instability of all-in-one intravenous feeding mixtures ('TPN' mixtures), which contain electrolytes, glucose, and amino acids in addition to the emulsion (Washington et al., 1989, 1990; Washington, 1990a–c). The unmixed emulsion is stabilized by a surface charge of −40 to −50 mV, which is developed near neutral pH by the surface ionization of minor components of the lecithin surfactant, predominantly phosphatidylserine and phosphatidic acid. Interaction of the emulsion with the electrolyte component of the TPN mixture leads to instability, as the ions in the mixture adsorb to the droplet, reducing the surface charge, and thus decreasing the collisional energy barrier to droplet coalescence.

The zeta potential of fat emulsions in electrolytes has previously been measured by laser doppler light scattering (Washington, 1990c). Unfortunately, it has proven difficult using this technique to study the zeta potential of the emulsion in anything other than dilute electrolyte solutions, which only provides a general indication of the possible surface chemistry of the droplets in TPN systems of higher ionic strength. In particu-

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lar, TPN mixtures contain 100–200 mM of monovalent electrolytes, and considerable ionized amino acid, and it has previously not been possible to measure zeta potentials in solutions of ionic strength greater than 30–40 mM. This has mainly been due to the high conductivity of these solutions, which causes them to carry high currents, and thus produces rapid sample heating during the electrophoresis experiment. Attempts to reduce the current with narrow-bore electrophoresis cells have not proven satisfactory in our hands, due to scattering from the cell walls. As a result, the general behaviour of fat emulsions in dilute monovalent and divalent electrolyte solutions is well-understood, but the application of these results to the study of real TPN mixtures requires considerable extrapolation; this has been a continued weakness of the approach.

We have recently acquired the latest generation of laser Doppler electrophoresis apparatus (Malvern Zetasizer 4), and this instrument has allowed us to perform measurements of emulsion zeta potential in real TPN environments of high conductivity. This is largely due to the improved electric field stability of this instrument over the previously used Zetasizer 2, which allows measurements to be made at much lower field strengths, and thus reduces sample heating significantly. In addition, the instrument has the ability to pulse the electric field at low duty cycle, thus reducing sample heating still further. As a result, it is now possible to measure electrokinetic parameters in 1:1 electrolytes of several hundred millimolar ionic strength, and it is straightforward to make measurements in complex TPN systems. As far as we are aware, these studies are the first systematic measurements of zeta potentials of fat emulsions in full-ionic-strength TPN environments that have been reported.

The use of light scattering techniques still requires that the disperse phase (the emulsion) be present in very low concentration in the TPN medium to avoid multiple light scattering effects. Consequently, this technique is not capable of measuring zeta potentials in TPN mixtures containing the normal proportion of fat emulsion (2–4% phase volume of lipid). Instead the TPN mixture must be compounded without the emulsion (but including the emulsions' water content), and small quantities of emulsion added to this mixture for study. Typically, disperse phase fractions of between 10^{-4} and 10^{-2}% are used. To a first approximation, the zeta potential thus measured will give a good indication of that in the TPN mixture containing a larger fraction of emulsion. However, it is possible that zeta potentials of fat emulsions in TPN mixtures are higher than those recorded in dilute emulsion systems, since the larger amount of emulsion in TPN systems has a large surface area, and may significantly deplete the electrolyte content of the continuous phase. This would make the apparent electrolyte activity in the TPN mixture lower than that calculated on the basis of its composition.

This effect has not previously been explored due to the difficulty of measuring zeta potentials at high disperse phase fractions by traditional methods such as moving-boundary techniques. A common method for studying this problem is to centrifuge the concentrated dispersion to isolate the equilibrium continuous phase, then resuspend a small amount of the colloidal material in this solution for the electrophoresis measurement. We do not consider this a useful method for the study of TPN mixtures, since our attempts to recover the equilibrium continuous phase in this way have led to samples contaminated with excess lecithin liposomes, even after extended ultracentrifugation.

A new technique for measuring zeta potential in concentrated dispersions has recently been introduced; this is electric sonic analysis, or ESA (Babchin et al., 1989; O'Brien, 1990). The method excites the sample with an alternating electric field of approx. 1 MHz frequency; this causes the charged droplets to oscillate in a similar manner, producing an ultrasound signal in the system which can be measured with an ultrasonic transducer. Briefly, the larger the droplet charge, the greater the amplitude of the ultrasound generated. Unlike laser Doppler techniques, the measurement is not absolute, and the instrument must be calibrated against a standard. This technique actually requires a significant fraction of disperse phase (typically 1–20%) in order to provide a usable signal strength. The current imple-

mentation (the Matec ESA 8000) is increasingly being used for the study of concentrated dispersions.

The present paper reports a study of the zeta potential of Intralipid 20% in TPN mixtures as the various components are added to the mixture. This allows the effect of each individual component to be studied, and the interactions between them to be assessed. In addition, we present preliminary data from the Matec ESA technique which allows the effect of increasing the emulsion phase volume on zeta potential to be studied. The results indicate that the two techniques in combination provide an almost complete model of electrokinetic effects in TPN mixtures, and represent a major advance over earlier studies from this laboratory.

Materials and Methods

Materials

Intralipid 20% (Kabi Pharmacia, batch no. 42320), Vamin 14 EF (Kabi Pharmacia, batch no. 90061), Vamin 9 glucose (Kabi Pharmacia, batch no. 90232), Glucose 10% (Baxter Health Care, batch no. 90JO8BD) and 30% (Baxter Health Care, batch no. 87L10A2) were obtained from the Hospital Pharmacy, Queen's Medical Centre, Nottingham. Sodium, calcium and ferric chlorides were analar grade from May and Baker.

The TPN mixtures studied by laser doppler light scattering were based on regimens recommended by Kabi Pharmacia. These were: *Regimen 1* – Vamin 14, 1000 ml; Glucose 30%, 1000 ml; Intralipid 20%, 500 ml. *Regimen 2* – Vamin 9 Glucose, 1000 ml; Glucose 10%, 1000 ml; Intralipid 20%, 500 ml.

In each case the amino acid and glucose solutions were mixed, and 400 ml of distilled water (representing the water content of the emulsion) was added. The zeta potential of Intralipid 20% was measured in these mixtures as a function of added monovalent and divalent electrolyte concentration (sodium and calcium chloride). In addition, we also measured the zeta potential of Intralipid 20% in varying dilutions of Vamin 14 (dilutions from 0 to 100% of the concentrated solution) and in sodium chloride solutions up to 0.6 M concentration.

Laser doppler light scattering

Zeta potentials were measured at 25°C using a Zetasizer 4 (Malvern Instruments, Malvern, U.K.). The most significant improvement to this instrument over earlier versions, for the purposes of the present work, is that the electrophoresis cell no longer uses semipermeable membranes to separate the sample from the driving electrodes. This results in a significant improvement in field stability. The ability of this instrument to pulse the driving current to reduce sample heating was also important. Zeta potentials were measured by diluting the emulsion into the continuous phase to a disperse phase fraction of approx. 10^{-5}. All measurements were made at a cell driving voltage of 50 V, which resulted in a field of 8 V cm^{-1}. The instrument automatically reduces the field duty cycle to limit the cell current to 20 mA. All measurements reported are the average of four separate determinations, and showed a standard deviation of approx. 2%. The dielectric constant of all the solutions was assumed to be 78, and their relative viscosity was measured by U-tube viscometry.

Electrosonic analysis

Electrosonic zeta potentials were measured at 25°C using a Matec ESA 8000 (Matec Applied Sciences, Hopkinton, MA). Approx. 200 ml of continuous phase was prepared with a disperse phase fraction of 5–10% of Intralipid 20% (i.e., 5–10% of lipid). The instrument requires calibration; a standard suspension of colloidal silica (Ludox) was used for this purpose. The samples were placed in the standard stirred sample container and the zeta potentials measured as the sample was titrated with calcium or ferric chloride. It was necessary to measure the response due to the electrolyte alone in a separate blank experiment; this ion resonance signal was subtracted from the signal due to the colloid. Note that all emulsion phase fractions specified here refer to the fraction of dispersed lipid in the system, i.e., a phase fraction of 10% corresponds to 10% lipid and not to 10% of a 20% emulsion.

Results

Light scattering in dilute emulsion systems

Fig. 1 shows the zeta potential of Intralipid 20% in increasing concentrations of sodium chloride up to 0.6 M as measured by light scattering. At low ionic strength the zeta potential was −55 mV, in agreement with that measured in previous studies with the Zetasizer 2. Increasing concentrations of sodium resulted in a rapid, apparently nonspecific, adsorption and reduction of charge, to approx. −4 mV at a sodium chloride concentration of 0.6 M.

Fig. 2 shows the effect of increasing concentrations of Vamin 14 on the zeta potential of Intralipid 20%. The amino acids reduced the surface charge to approx. −3 mV in full-strength Vamin 14, with no evidence of charge reversal. Most TPN mixtures typically contain 30–40% of this solution, which would depress the zeta potential to approx. 10 mV in the absence of added electrolytes.

Figs. 3 and 4 illustrate the effect of adding electrolytes on the zeta potential of Intralipid 20% in TPN regimen no. 1. In the absence of added electrolytes, the zeta potential was −7 mV. The corresponding point on the Vamin 14 curve (Fig. 2) was −8 mV, indicating that within

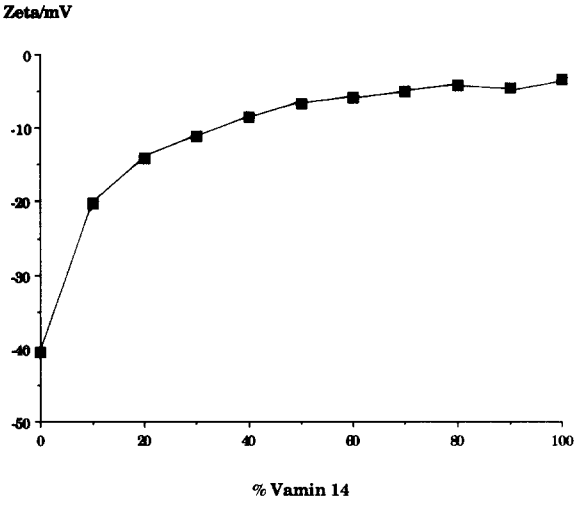

Fig. 2. Zeta potential of Intralipid 20% in various concentrations of Vamin 14 EF.

experimental error, this potential was determined by the amino acid alone, and was not influenced by the presence of glucose. Adding sodium (0–80 mM) caused the zeta potential to be gradually reduced to about −4 mV (Fig. 3). Addition of calcium (0–15 mM) caused a more rapid charge reduction with a point of zero charge (pzc) at 7 mM calcium, followed by charge reversal. In the presence of 50 mM sodium chloride, the pzc with

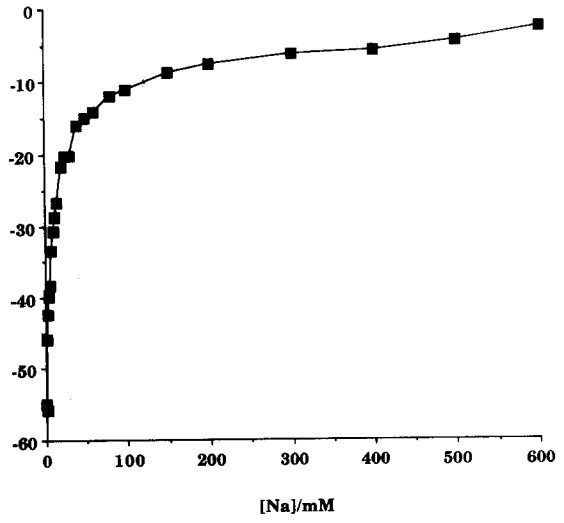

Fig. 1. Zeta potential of Intralipid 20% at pH 7 as a function of sodium concentration.

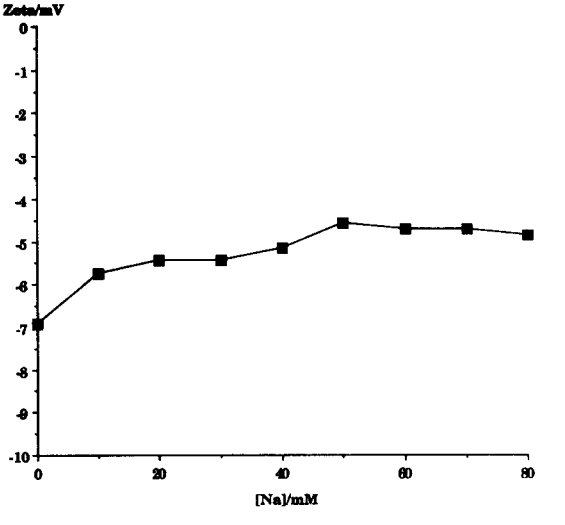

Fig. 3. Zeta potential of Intralipid 20% in TPN model system 1 as a function of added sodium chloride concentration.

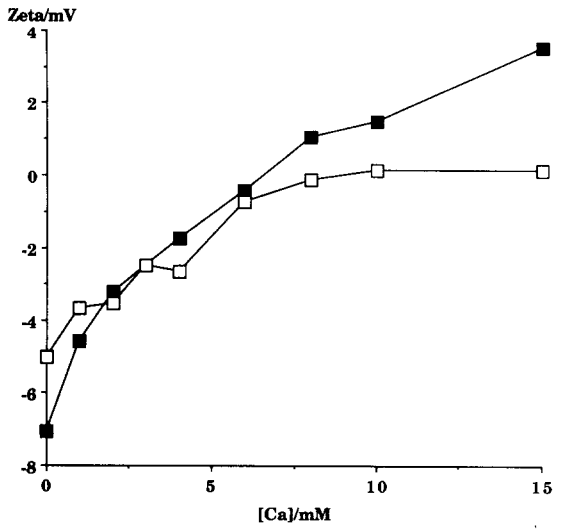

Fig. 4. Zeta potential of Intralipid 20% in TPN model system 1 as a function of added calcium chloride concentration, (□) with and (■) without 50 mM sodium chloride.

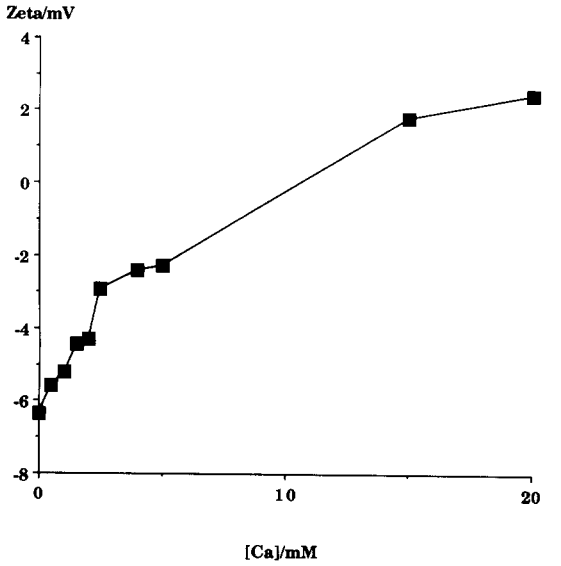

Fig. 6. Zeta potential of Intralipid 20% in TPN model system 2 as a function of added calcium chloride concentration.

calcium was shifted to 9 mM and the charge reversal was much less pronounced, within 1 mV of zero.

Similar trends were observed in TPN regimen no. 2. Addition of sodium (Fig. 5) caused the potential to fall from −7.5 mV (0 mM added sodium) to −5.5 mV (50 mM added sodium). These changes occurred on a base level of 29 mM monovalent ion and 1.65 mM divalent ion, contributed by the Vamin 9 glucose. Addition of calcium (Fig. 6) caused charge reversal with a pzc at 10 mM calcium.

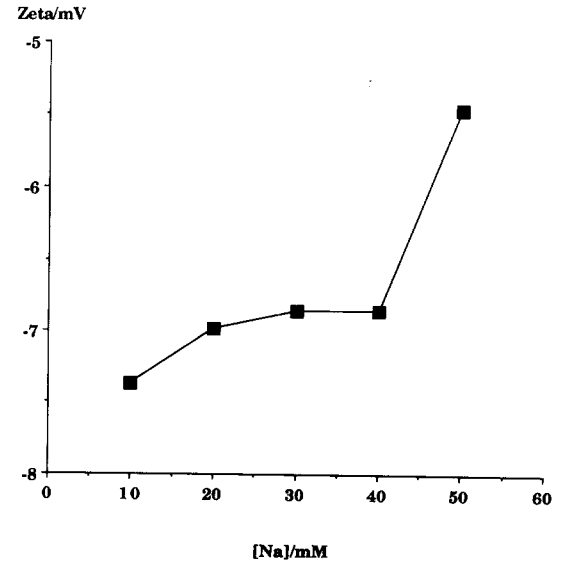

Fig. 5. Zeta potential of Intralipid 20% in TPN model system 2 as a function of added sodium chloride concentration.

Electrosonic analysis in concentrated emulsion systems

The zeta potentials determined by electrosonic analysis at higher emulsion phase fractions were broadly in line with those in dilute emulsion systems determined by light scattering. Fig. 7 shows the zeta potential of Intralipid 20% at pH 7 as a function of emulsion phase fraction; this decreased from −37 mV at 1% lipid phase fraction, to −19 mV at 10% phase fraction. The effect of adding calcium to an emulsion of 10% phase fraction is depicted in Fig. 8. The potential decreased from −25 mV in the absence of calcium to a pzc at approx. 3.5 mM calcium, followed by charge reversal of +2.4 mV in 8 mM calcium (note that these figures are those obtained after the subtraction of the ion resonance signal). Finally, the addition of ferric iron (0–0.5 mM) caused rapid charge reversal with a pzc at approx. 70 μM Fe^{3+}. However, this effect was

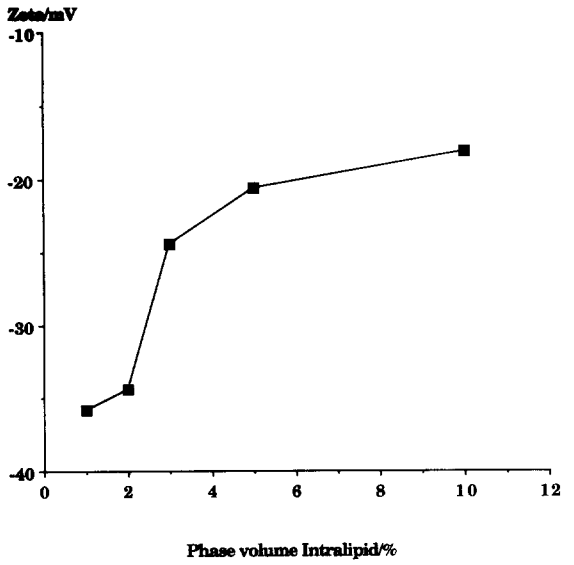

Fig. 7. Zeta potential of Intralipid 20% as a function of disperse phase fraction at pH 7 by ESA.

not as pronounced as that measured in dilute emulsion systems using light scattering, in which a pzc was obtained at approx. 30 μM ferric iron, and charge reversal occurred much more rapidly.

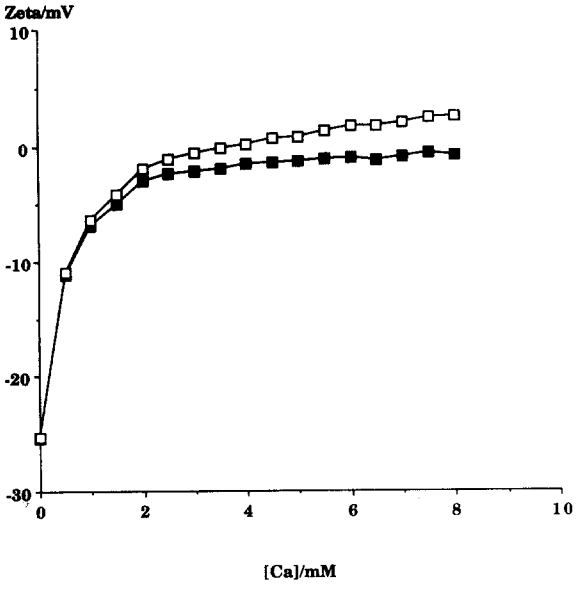

Fig. 8. Zeta potential of Intralipid 20% as a function of calcium chloride concentration by ESA. (■) Raw data, (□) data after subtraction of ion resonance signal.

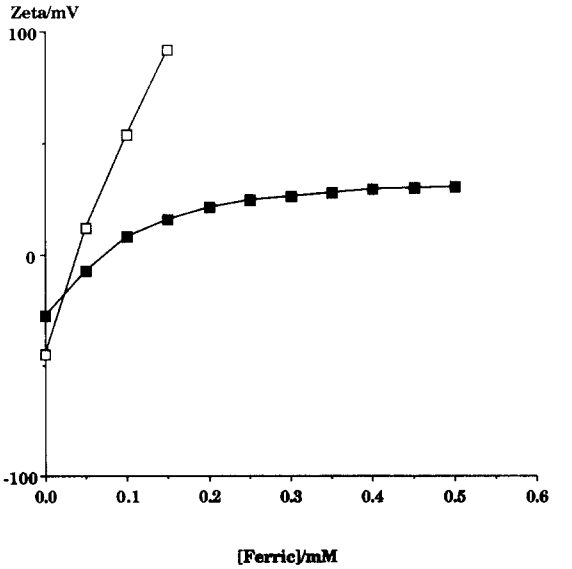

Fig. 9. Zeta potential of Intralipid 20% as a function of ferric ion concentration. (■) In 10% colloid phase fraction by ESA, and (□) in dilute colloidal system by light scattering.

Discussion

The results demonstrate the importance of composition in the determination of zeta potential in TPN mixtures, and support results derived previously using dilute systems. The absorption of sodium (Fig. 1) appears to follow the Gouy-Chapman equation, and in fact this data set can be fitted with high precision using only the surface charge density as a variable parameter. A value of 2.7% ionized surface lipid was obtained by this approach. However, it is possible also to fit the data set accurately by assuming a higher value of lipid ionization and weak specific adsorption of sodium to the surface. In fact, a better fit will always be achieved using this latter model, since it introduces an extra parameter – the binding strength of the monovalent ion to the surface. Consequently, these data cannot be used to unequivocally prove or disprove the binding of monovalent ions to the emulsion droplet. We have always stressed that this weak binding would be of importance in TPN systems, since the concentration of monovalent ions is relatively high in TPN mixtures, and since the competitive effects of ion adsorption seen in previous dilute emulsion systems (Washington, 1990a) cannot be ex-

plained without invoking weak specific adsorption of monovalent ions. This weak adsorption of monovalent ions to phospholipid surfaces is already known from NMR studies of liposomal bilayer systems (Eisenberg et al., 1979).

Similar arguments can be applied to the effects of amino acids on zeta potential. Vamin 14 reduced the droplet charge without demonstrating any charge reversal effects, but it is at present difficult to say whether or not any specific adsorption of amino acids was taking place, or whether the effects were those of completely indifferent ions. It would be surprising if the more hydrophobic amino acids, such as tryptophan or tyrosine, did not adsorb to the droplets to a certain extent. However, a detailed discussion of such data is complicated by the numerous components present in the amino acid solution, and will have to await more extensive studies. However, it does indicate that amino acids not only stabilize by calcium binding and Hamaker effects (Washington et al., 1992), but also destabilize by reducing interdroplet charge, and the overall effect on TPN stability will be a combination of these two factors.

The adsorption of ions in model TPN systems demonstrated similar effects to those seen in simple electrolyte solutions in previous studies. Monovalent ions had little effect on the zeta potential, possibly due to competition with amino acids and due to the charge-screening effect of the latter. Divalent ions (Figs 4 and 6) exerted a more pronounced effect, and showed that a number of TPN mixtures with high divalent electrolyte concentration could be expected to be charge reversed. As in dilute systems, the presence of monovalent ions caused the pzc due to the divalent ion to be shifted to higher concentrations; we interpret this as being due to competition between the ions for surface binding, emphasizing the importance of even weak specific adsorption for monovalent ions. If it is desired to stabilize a high electrolyte system by charge reversal, it is evident that a low monovalent to divalent ion ratio must be used. The charge reversal caused by 15 mM calcium in TPN system 1 was almost completely abolished by 50 mM of added sodium chloride.

The effect of increasing the phase volume of the emulsion can be studied using the electrosonic analysis method (Figs 7–9); the zeta potential of 1% phase fraction emulsion at pH 7 was approx. −37 mV, and this declined to −19 mV at an emulsion phase fraction of 10%. The discrepancy between the low phase volume measurement and that previously obtained by electrophoresis (−40 to −50 mV) is not surprising considering the very different nature of the two measurement systems; indeed the broad agreement is gratifying. The reduction of zeta potential with increasing phase fraction is almost certainly only apparent; as the concentration of emulsion droplets increases, the measured mobility will fall due to retardation in the electric field of adjacent droplets. The surface potential was probably little altered, but the mobility was decreased by these interdroplet interactions. This phenomenon warrants further study since it may be of importance in determining the stability of the unmixed emulsion systems.

The titration of Intralipid at 10% disperse phase fraction with calcium (Fig. 8) showed a very similar behaviour to that now familiar from light scattering. The absolute magnitudes of the zeta potentials measured were approximately half those observed by light scattering, presumably due to mobility retardation, and the pzc at a calcium concentration of 3–3.5 mM was in good agreement with the previous value of 3 mM (Washington, 1990c). In order to obtain this value it was necessary to perform a blank experiment to determine the instrument response to the ions added; this was then subtracted from the response in the colloidal system. This background corresponded to a zeta potential of only 3 mV at the highest calcium concentration, and so the correction does not seem unreasonable; however, the correction in stronger electrolyte systems, such as TPN mixtures, was extremely large and may amount to several times the signal from the colloid. Under these conditions this background subtraction is questionable, which unfortunately limits the applicability of this technique to the study of TPN mixtures directly.

A significant problem which arises when extrapolating dilute colloid studies to more concen-

trated colloid systems such as TPN mixtures is that it is difficult to estimate the extent of depletion of the electrolyte from the continuous phase by adsorption to the larger surface area of the colloid in the more concentrated system. We would expect that a large amount of colloid would have sufficient surface capacity to adsorb a significant fraction of an ion from solution, thus decreasing its bulk concentration. This would cause the surface adsorption to be lower in concentrated colloidal systems than in dilute systems; in effect it is necessary to 'fill up' all the surface sites before a particular concentration of ion in the continuous phase can be achieved. The magnitude of this effect has not been previously determined; however, it is straightforward to study by the ESA technique. The agreement of the calcium adsorption isotherm of Fig. 8 with the earlier results in dilute colloid systems indicates that the effect is not of sufficient magnitude to be detectable in TPN systems containing up to 10% of emulsion. Since most TPN systems contain 2–3% disperse lipid, it appears that previous studies performed using dilute colloid systems can be extrapolated to TPN systems without having to make direct allowance for ion depletion by absorption.

Absorption depletion will become more evident as more strongly binding ions are studied at lower concentrations. Thus it is evident from the ferric ion binding isotherm (Fig. 9) that this ion was so strongly absorbed in TPN systems that the bulk phase was considerably depleted, and so the charge-reversal in systems containing larger amounts of colloid was much weaker than in the dilute systems. It would appear to be necessary to add several millimoles of ferric ion to fill the sites before a significant bulk phase concentration can be achieved. The strong absorption is a consequence of the Schultze-Hardy rule, which states that the adsorption of nonspecifically binding ions to a surface will be in proportion to the sixth power of the ion charge.

Conclusions

The present investigation demonstrates that it is possible to study the electrokinetic properties of fat emulsions in real TPN systems, rather than having to extrapolate studies in dilute emulsion and electrolyte systems. It is particularly pleasing that the general principles of ion adsorption appear to be similar in both the dilute model systems and in real TPN systems, so that previous hypotheses concerning TPN behaviour will not have to be revised. The ability to make these measurements will allow the stability of individual TPN mixtures to be compared to their electrokinetic properties, which will be a powerful tool in the resolution of the TPN stability problem.

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