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The electrokinetic properties of phospholipid-stabilized fat emulsions. II. Droplet mobility in mixed electrolytes

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Summary

The paper develops a semiempirical model which describes the electrokinetic behaviour in simplified form of fat emulsion droplets in mixed electrolyte solutions. The electrolyte-droplet interaction is described using a combination of specific adsorption to sites on the droplet, and nonspecific effects described by the Gouy-Chapman theory. The extensions to the theory required for an understanding of the stability of TPN mixtures are discussed.

Introduction

Intravenous feeding emulsions consist of vegetable triglycerides emulsified in water by lecithin, and are normally administered to patients as an admixture also containing electrolytes, amino acids and carbohydrates. The instability of such mixtures, due to the interactions of electrolyte and charged emulsion droplet, is well-known (see e.g. Ailwood, 1984; Davis et al., 1985). The theoretical modelling of the stability of TPN mixtures would appear to be a straightforward application of classical electrokinetics and the DLVO theory of colloid stability. Unfortunately the large number of components present in a parenteral nutrition mixture makes the development of an electrokinetic model very complex. This complexity is compounded by the heterogeneous nature of the emulsifier, which contains both neutral and negatively charged phosphatides. Recently Washington et al. (1989) reported measurements of the electrophoretic mobility of model parenteral nutrition emulsions which used mixtures of purified phospholipids as emulsifiers. Since these materials are well-characterised, it is possible to describe their behaviour, at least approximately, in terms of the classical electrokinetic theory. This provides a basis for the description of real emulsion systems using natural lecithin emulsifiers.

The present paper describes a semiempirical theory which allows the accurate modelling of droplet zeta potential in mixed electrolyte systems. Although this departs to some extent from a rigorous electrokinetic description, the approximations made appear to be justified on the basis of the

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good agreement obtained with experiment. The effects of amino acids, carbohydrates or pH on the system stability have not yet been accounted for, and so it is not yet possible to predict the stability of a TPN mixture on an ab initio basis. However, it appears likely that extensions to the theory will allow the description of these effects in the near future.

The electrophoretic properties of phospholipids themselves and their interactions with electrolytes are well understood from studies of liposomal model systems. All phospholipids bind both monovalent and divalent electrolytes specifically (McLaughlin et al., 1978; Van Dijck et al., 1978; Eisenberg et al., 1979; Lau et al., 1981; Altenbach and Seelig, 1984; Macdonald and Seelig, 1987). For our purposes it is convenient to use the definition of specific binding given by Mohliner (1966); "there is specific adsorption if the experimental data cannot be explained by the theory of the diffuse double layer". By this definition, the binding of monovalent cations is much weaker than the binding by divalent cations, and the binding by neutral phospholipids (e.g. phosphatidylcholine, PC) is much weaker than the binding of acidic phospholipids (e.g. phosphatidylglycerol, PG). Consequently the binding of e.g. calcium or magnesium by PG or phosphatidylserine (PS) is the major interaction and provides a qualitative understanding of the processes involved. However, it is not sufficient to consider this interaction alone if a quantitative description is required. This is largely due to the fact that the more weakly interacting materials (e.g. sodium and phosphatidylcholine) are present in much greater amounts than the strongly interacting ones. For example, a typical TPN mixture contains 150 mmol of sodium; Eisenberg et al. (1979) quote a first-order association constant between PS and sodium of 0.6 M^{-1}, implying that approximately 25% of available binding sites will be filled with sodium if a bulk concentration of 150 mmol is present in the continuous phase.

The theory to be described is based on an absorption site model, in which a cation can associate with a site of unspecified nature on the droplet surface. The interaction of phospholipids with divalent cations is thought to proceed through the production of a 1 : 2 ion/lipid complex, but an explicit description of the stoichiometry of the site is not used in the present model. This provides an advantage over a more rigorous treatment (e.g. the adsorption of cations to PC/PG liposomes; Macdonald and Seelig, 1987) in that the number of potentially variable parameters is reduced. In practice Macdonald and Seelig demonstrated that an explicit description of the binding site stoichiometry does not significantly influence the behaviour of the model until extremely high concentrations of strongly binding cations are achieved (e.g. 100 mM calcium). At this concentration the binding site model is inappropriate since the calcium interacts strongly with the phospholipids, causing lateral phase separation and perturbation of the statistical distribution of binding site units. These concentrations of strongly binding ions are not normally found in TPN mixtures.

Theoretical

The object of the theory is to provide an algorithm or analytic procedure which allows the mobility of the emulsion droplets in a mixed electrolyte to be predicted from measurements of their behaviour in individual electrolytes. The starting point is a description of the binding of a set of ions to sites on the droplet surface.

Suppose there exists a total surface concentration of binding sites, [s], which can associate reversibly with a series of i ions $I_0 \ldots I_i$ in a first-order manner. The concentration of the ith ion is denoted $[I_i]$. The equilibrium is given by:

$$I_i + S \Leftrightarrow SI_i,$$

where the binding equilibrium constant, K_i is given by:

$$K_i = \frac{[SI_i]}{[I_i][S]}.$$

[S] is the concentration of unoccupied binding sites and is given by:

$$[S] = [s] - \sum_i [SI_i].$$

By selecting an ion I_j and eliminating the unknown concentration of free sites [S] it is straightforward to show that the fraction of sites, f_j, occupied by the ion I_j is given by:

$$f_j = \frac{[SI_j]}{[s]} = \frac{K_j[I_j]}{1 + \sum_i K_i[I_i]}.$$

This describes the fraction of sites filled by each binding ion. The next stage is to calculate the effect that each of these has on the zeta potential. This is straightforward if the stoichiometry of the binding sites is known, which is not necessarily the case. Additionally the fact that all the lipids present on the droplet surface, including phosphatidylcholine, bind cations to some extent, leads to ill-definition of the binding site. Rigorously, each type of site should be treated as having a separate K_i for a given ion; however this would lead to a large number of additional parameters, and necessarily more accurate data would be required for their unambiguous determination. This can be overcome with an empirical description as follows. The zeta potential is described as the weighted mean of the potential in the absence of ion binding (which is just the zeta potential of the emulsion alone) and the potential when all sites are filled by the ion. This is the potential measured at infinite electrolyte concentrations, although of course this potential need not (in fact cannot) be determined in an explicit experiment; it is found from fitting the zeta potential vs. electrolyte concentration curve. If the zeta potential of the droplet in the absence of electrolytes is denoted by ζ_0 and that of the droplet with all sites filled by the ion I_i is ζ_i, then the droplet zeta potential, ζ, can be estimated as:

$$\zeta = \zeta_0\left(1 - \sum_i f_i\right) + \sum_i f_i\zeta_i.$$

This is an empirical description, since rigorously the surface should be described additively in terms of its charge density, σ, and the surface potential ψ_0 and the zeta potential determined by the Gouy-Chapman theory. This requires the introduction of an extra parameter, the distance of the hydrodynamic shear plane from the surface, as well as the calculation of the Debye-Huckel length in the solution. Although this is straightforward the extra complexity is difficult to justify in a phenomenological model since experiments in this direction were not found to significantly improve the fitting accuracy of the data.

The model thus describes the binding of each ion, and its effect on the zeta potential, by two parameters which have three important characteristics. Firstly, they have physical significance; K_i describes the binding strength and ζ_i describes the zeta potential when the sites are filled. Secondly, both parameters can be obtained accurately from measurements on a well-defined system (emulsion in a single electrolyte). Finally, in practice the two parameters prove to be neither redundant nor correlated, which ensures that their estimation is not an ill-conditioned problem.

Finally it must be noted that the concentration of each ion driving its adsorption is not the bulk concentration $[I_i]$, but the concentration at the droplet surface, $[I_{i,s}]$ which is different due to the repulsion or attraction of the ion from the charged interface. This can be found from the Boltzmann equation:

$$[I_{i,s}] = [I_i] \exp\{-z_i e\zeta/kT\},$$

where z_i is the charge on the ion I_i.

The introduction of the surface concentration term complicates the modelling since it causes the concentration to be a function of zeta potential; the zeta potential is already a function of surface concentration, and so an analytic solution of these equations proves difficult. Stable solutions were found by an iterative method which accepts trial values of the binding parameters and calculates the resulting zeta potential as a function of the concentration of the electrolytes. This allows the estimates of the parameters to be refined until the best (least-squares) agreement with the experiment is obtained. The theory was tested by finding the binding parameters for single electrolytes and then using these to predict the behaviour in a mixed electrolyte system.

Materials and Methods

Intralipid 20% (Batch No. 37553) was obtained from the hospital pharmacy, Queen's Medical Centre, Nottingham. Sodium and calcium chlorides were analar grade from May & Baker. Zeta potentials were determined using a Malvern Zetasizer II. All computation was performed in Microsoft Basic on a Macintosh SE.

Results

Figure 1 shows the charge reversal of Intralipid 20% by calcium in the range 0–10 mM, and the best fit to the data. This was obtained at a value of K_{Ca} of 600 mol^{-1} and ζ_{Ca} of +25 mV, i.e. the sites were half filled with calcium at 1/600 M (1.67 mM), and the zeta potential of the droplets at 'infinite' calcium concentration was +25 mV. It should be stressed that ζ_{Ca} is only a parameter of the model and is not directly measurable, since multiple ion adsorption and anion effects would rapidly become significant at high electrolyte concentrations.

Figure 2 shows a similar data set for the adsorption of sodium in the concentration range 0–40 mM on to Intralipid 20%. Since sodium is considered not to specifically adsorb to droplet surfaces, and to neutralize droplet charge at high concentrations, ζ_{Na} was assumed to be zero. The best fit was obtained with a value of K_{Na} of 15 mol^{-1}.

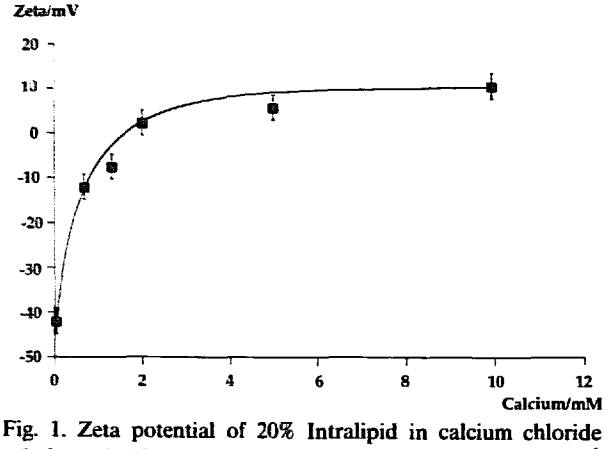

Fig. 1. Zeta potential of 20% Intralipid in calcium chloride solutions (0–10 mM). Solid line is fit with $K_{Ca} = 600$ mol^{-1} and $\zeta_{Ca} = +25$ mV.

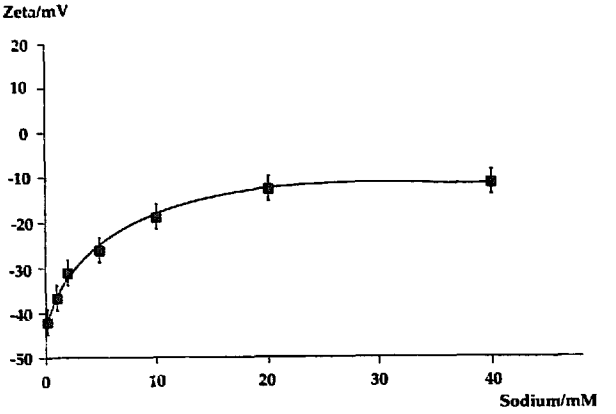

Fig. 2. Zeta potential of 20% Intralipid in sodium chloride solutions; (0–40 mM). Solid line is fit with $K_{Na} = 15$ mol^{-1} and $\zeta_{Na} = 0$.

Using these values it was possible to predict the zeta potential in a mixed electrolyte solution and compare the values obtained with experiment. This is done in Fig. 3, which is the zeta potential of Intralipid 20% in calcium chloride solutions containing 40 mM sodium chloride.

Discussion

The model developed appears to provide reasonable agreement with the experimental data for the limited range of systems studied to date. It is particularly pleasing that the model gave a reasonable degree of prediction in mixed systems

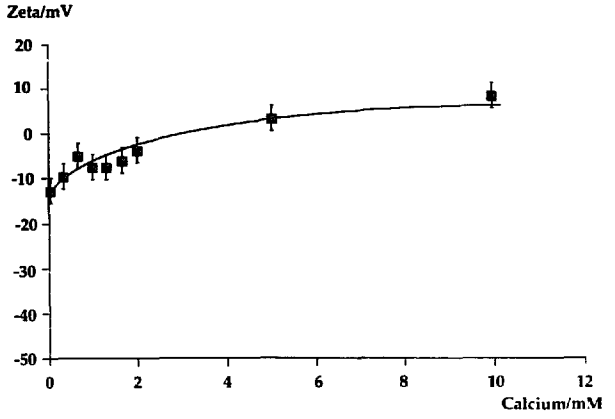

Fig. 3. Zeta potential of 20% Intralipid in calcium chloride solutions (0–10 mM) in the presence of 40 mM sodium chloride. Solid line is prediction with $K_{Ca} = 600$ mol^{-1}, $\zeta_{Ca} = +25$ mV, $K_{Na} = 15$ mol^{-1} and $\zeta_{Na} = 0$.

(Fig. 3) when the adsorption parameters were determined in single electrolyte solutions.

A model of TPN zeta potential such as that described here has two functions. Firstly it allows a large quantity of data gathered from a wide range of system compositions to be rationalized in terms of a small number of parameters. Input to the model is the zeta potential of emulsion in any electrolyte mixture which the investigator (or emulsion manufacturer) can measure accurately. This allows the second function to be realized, i.e. that the zeta potential in an electrolyte mixture not previously investigated can be predicted without measurement. This approach is illustrated in the results presented here. The input data, zeta potentials of emulsion in solutions of sodium or calcium chloride, allows the adsorption parameters of sodium and calcium to be determined. The potential in mixtures of these two electrolytes can then be predicted. It must be stressed that the curve in Fig. 3 is a prediction using the binding parameters obtained from the data in single electrolyte solutions, and has not itself been explicitly fitted.

Models of this type offer some hope of attaining a predictive understanding of the stability of parenteral nutrition mixtures. In order to accomplish this two further steps are required. Firstly, the influence of the remaining components (amino acids and carbohydrates) on emulsion zeta potential must be understood. It will then be possible to predict zeta potentials in real TPN mixtures.

Secondly, it is necessary to understand in detail the relationship between zeta potential and emulsion stability. The central nature of the critical flocculation concentration in this problem is well known. The overall relationship between zeta potential and fat emulsion stability has been studied closely (see e.g. Washington et al., 1989); it appears that a critical zeta potential is required for stability, which is the zeta potential at the critical flocculation concentration. However, predicting this critical zeta potential is more difficult. It appears that it is approximately ± 12 mV in most Intralipid 20% samples in simple electrolytes; however the pure phospholipid emulsions studied previously have a critical zeta potential of ± 25 mV, for which there is no obvious explanation. In addition, zeta potentials in practical TPN mixtures are very low; preliminary experiments suggest that they rarely exceed ± 3 mV, even in systems which show little flocculation or droplet coalescence over periods of several months. It is possible that amino acids may confer stability on TPN mixtures not by influencing zeta potential but by reducing the critical zeta potential in the mixture. Experiments to test this hypothesis are in progress.

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