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The electrokinetic properties of phospholipid-stabilized fat emulsions.
III. Interdroplet potentials and stability ratios in monovalent electrolytes

C. Washington

Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham (U.K.)

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Summary

The interdroplet potential in a typical parenteral fat emulsion in the presence of sodium ions has been estimated from electrolyte stability data in a conventional manner as a sum of electrostatic, Van der Waals, and hydration forces. The hydration forces are important at all electrolyte concentrations, and result in the abolition of the primary potential minimum. At high (> 100 mM) electrolyte concentrations a secondary minimum is observed into which the droplets can flocculate reversibly. The stability ratios of the emulsion in monovalent electrolyte solutions were calculated from the interdroplet potentials. Good agreement was found between the calculated values and those measured by turbidimetric means. Optimization of the model yielded a value for the Hamaker constant (A_{11}) of soya oil of 4.75×10^{-20} J ($A_{121} = 6.5 \times 10^{-22}$ J in water). The importance of the hydration forces in determining the interdroplet potential suggests that these should receive at least as much attention as the electrostatic forces in the consideration of emulsion stability. It is suggested that amino acids exert their stabilizing effect in parenteral feeding mixtures by modifying the hydration forces, possibly allowing them to be more efficiently propagated through the solvent medium. The results offer hope that it may ultimately be possible to predict the stability of TPN mixtures from their composition alone.

Introduction

The stability of parenteral fat emulsions in electrolyte solutions is a widely explored problem. These emulsions are stabilized electrostatically by virtue of the phospholipid emulsifier used, and many previous workers have rationalized their stability using the classical DLVO theory. The forces between the droplets are considered as a sum of a repulsive electrostatic part and an attractive Van der Waals part, which provides a good semi-quantitative description with a minimum of mathematical complexity.

However, in recent years there has been an increasing awareness that additional forces need to be considered between the droplets due to solvent structure at the droplet interface. These forces are particularly important at short range and tend to decay exponentially into the solvent

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

medium. Fortunately, the magnitude of these forces for phospholipid-coated surfaces has already been investigated by elegant thermodynamic studies on liposomes (Lis et al., 1982) and so their contribution to the interdroplet potentials of fat emulsions can be calculated in a straightforward manner.

The primary object of studying the interaction potential of the emulsion in a TPN mixture is to evaluate the stability of the disperse phase to flocculation. Ultimately, one would wish to produce an estimate of emulsion stability ratio in a complex medium containing electrolyte, amino acids, glucose, and the many minor but doubtless important components which are routinely added by the pharmacist. The stability of a colloidal system can be calculated from the potential functions using the diffusion theory of Fuchs (1934) or the kinetically based procedure of Marmur (1979). The latter is particularly appropriate in this system since it specifically takes into account the possibility of flocculation into a reversible shallow secondary energy minimum with no energy barrier. Under these circumstances, Fuchs' equation has not been found to provide satisfactory results, since it is primarily intended to quantify irreversible flocculation into the primary minimum over a potential energy barrier.

In order to evaluate the interdroplet potentials, a study was made of the simplest emulsion-electrolyte system, that in which flocculation is induced by a suitable concentration of a nonspecifically absorbing monovalent electrolyte. The measured stability ratios can be compared to those calculated from suitable trial potential functions, allowing the true potential function to be estimated.

Theoretical

The total interdroplet potential energy V_T is the sum of contributions from electrostatic repulsion V_R, Van der Waals attraction V_A, and solvent (hydration) repulsion V_H:

$$V_T = V_R + V_A + V_H \tag{1}$$

Throughout this paper dimensionless reduced energies are used for convenience, i.e. energies in units of kT. A number of approximate expressions are available for the electrostatic repulsion V_R depending on the ionic strength and potential; that developed by Verwey and Overbeek (1948) is applicable at high κa and low surface potential:

$$V_R = 2\pi\epsilon_0\epsilon_r a \Psi_S^2 \ln(1 + \exp(-\kappa H)) \tag{2}$$

Here Ψ_S is the surface or Stern potential, H is the surface separation between the interacting droplets, a is the particle radius, ϵ_0 is the permittivity of free space, and ϵ_r is the relative permittivity of water.

The Debye-Hückel parameter κ is conveniently given by

$$\kappa = 3.288\sqrt{I}\ (\text{nm}^{-1}) \tag{3}$$

in an aqueous system at 25°C. I is the ionic strength of the electrolyte solution.

The Stern potential is normally calculated from the zeta potential using the Gouy-Chapman theory. The potential $\Psi(x)$ decays away from the particle surface with distance x as:

$$\Psi(x) = \frac{2kT}{e} \ln\frac{1 + \alpha \exp(-\kappa x)}{1 - \alpha \exp(-\kappa x)} \tag{4}$$

where α is given by

$$\alpha = \frac{\exp(e\Psi_S/2kT) - 1}{\exp(e\Psi_S/2kT) + 1} \tag{5}$$

T is the absolute temperature, k is Boltzmann's constant and e is the electronic charge. The zeta potential ζ is the potential at the surface of hydrodynamic shear, which is normally taken to be 0.2 nm in phospholipid systems (Eisenberg et al., 1979).

The zeta potential cannot unfortunately be measured at the high electrolyte concentrations used here, due to the high solution conductivity, so it must be calculated by extrapolation from measurements at lower electrolyte concentrations. This is done firstly by finding the surface charge density σ_0 at the lower electrolyte concentrations using the Gouy equation:

$$\sigma_0 = 11.74c^{0.5}\sinh(19.46z\Psi_0)\ (\mu\mathrm{C\ cm^{-2}}) \qquad (6)$$

Here c is the electrolyte concentration and z is the charge on the adsorbed ion (1 in the case of sodium). For this purpose Ψ_S is taken to be equal to the measured value of ζ, since very little potential decay will occur at low electrolyte concentrations ($1/\kappa \gg 0.2$ nm). Having calculated σ_0, Ψ can be obtained at any higher electrolyte concentrations. This assumes that no specific binding of the electrolyte takes place, a hypothesis which is supported by the study of Gamon et al. (1989).

The Van der Waals interaction potential V_A is given by (Schenkel and Kitchener, 1960):

$$V_A = \frac{A_{121}a}{12H(1 + 11.12H/l)} \qquad (7)$$

This expression allows empirically for the effects of retardation through the characteristic oscillator wavelength l, which has been taken to be 100 nm. In practice retardation is not of major importance at small particle separations. The constant A_{121} is the Hamaker constant of soya oil (medium 1) in water (medium 2). Normally, this is treated as a variable parameter, since it is difficult to measure experimentally; however, to provide an initial value, the equation of Fowkes (1967) for the Hamaker constant A_{11} in vacuo can be used:

$$A_{11} = \frac{2\pi d^2\gamma_d}{\Lambda} \qquad (8)$$

where d is the spacing of atomic centres (0.46 nm. for organic liquids), Λ is a constant with a value of approx. 0.9, and γ_d is the dispersion contribution to the surface tension. This can be considered to be equal to the measured surface tension for liquids of low dipole moment, but it is often more straightforward to calculate it from the refractive index using the empirical relation of Papazian (1971):

$$\gamma_d = 286\left[(n^2 - 1)/(2n^2 + 1) - 0.1\right] \qquad (9)$$

The Hamaker constant in a suspending medium (A_{121}) is the related to the disperse phase Hamaker constant A_{11} and the continuous phase Hamaker constant A_{22} by:

$$A_{121} = \left((A_{11})^{0.5} - (A_{22})^{0.5}\right)^2 \qquad (10)$$

The hydration potential V_H was calculated using the semiempirical expression:

$$V_H = \frac{\pi a\lambda^2 P_0}{kT}\exp(-H/\lambda) \qquad (11)$$

where the values for the amplitude and decay constants P_0 and λ were taken to be equal to those for egg phospholipid vesicles (Lis et al., 1982). Since the degree of surface hydration is determined by phospholipids, this is not likely to be a large source of error, although the lipid head-group spacing in the monolayer at the droplet surface may be slightly different from its bilayer value.

The total potential was evaluated as the sum of these separate contributions and the depth of the secondary minimum Φ_{min} was found. The fraction of droplets f_s in the secondary minimum is given by (Marmur, 1979):

$$f_s = \frac{4}{\pi^{1/2}}\int_0^{(-\Phi_{min})^{1/2}} x_1^2\exp(-x_1^2) \cdot \left[\mathrm{erf}(x_s) - \frac{2}{\pi^{1/2}}x_s\exp(-x_s^2)\right]\mathrm{d}x_1 \qquad (12)$$

where

$$x_s = \left(-\Phi_{min} - x_1^2\right)^{1/2} \qquad (13)$$

(all energies in units of kT)

The stability ratio W in the absence of flocculation into the primary minimum is:

$$W = 1/f_s \qquad (14)$$

Using these latter two equations, it is possible to find W as a function of Φ_{min} alone. This is plotted in Fig. 1. The values obtained are realistic since, at low Φ_{min}, the flocculated state is weakly bound

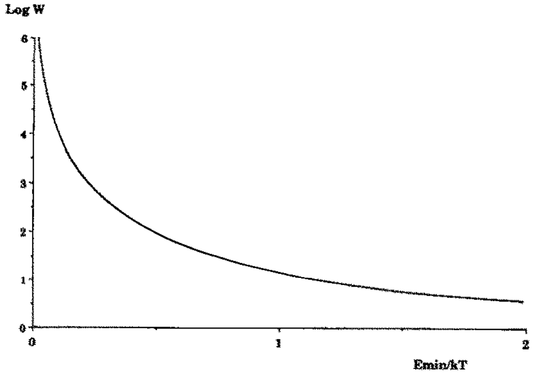

Fig. 1. Stability ratio vs potential energy minimum depth calculated from the Marmur equation.

and the available thermal energy is sufficient to disperse it; thus, the emulsion is stable. When $\Phi_{\min}$ is large (i.e. of the order of kT), however, the flocculated state is significantly bound and the emulsion stability ratio is low.

The formation rate of doublets k_{11} is calculated from the initial turbidity τ_0 and the initial rate of increase of turbidity $(\mathrm{d}\tau/\mathrm{d}t)_0$ by:

$$k_{11}=\frac{(1/\tau_0)(\mathrm{d}\tau/\mathrm{d}t)_0}{FN_1} \tag{15}$$

F is an optical factor arising from Rayleigh-Gans-Debye scattering theory and has been tabulated by Lichtenbelt et al. (1974). N_1 is the number concentration of droplets, calculated from the phase volume and the PCS mean diameter, assuming a monodisperse system (this is not strictly correct but is the best approximation available).

The stability ratio is defined as the ratio of the flocculation rate to the diffusion-controlled or Smoluchowski (1917) rate, given by:

$$k_{\mathrm{D}}=\frac{8kT}{3\eta} \tag{16}$$

i.e.

$$W=\frac{k_{\mathrm{D}}}{k_{11}} \tag{17}$$

Materials and Methods

The flocculation rate of Intralipid 20% (ex. Hospital Pharmacy, Queen's Medical Centre, Nottingham) was measured in sodium chloride solutions of 0.1–0.5 M concentration using a turbidimetric method described previously (Washington and Davis, 1987). The zeta potential of Intralipid 20% was measured in sodium chloride solutions (0–40 mM) by Malvern Zetasizer at pH 7 and 25 ° C (Washington, 1990). The mean droplet diameter was taken as the PCS z-average diameter from measurements with a Malvern K7025 correlation spectrometer. The refractive index of a sample of soya oil (J. Sainsbury PLC) was determined by Abbé refractometer at 20 ° C.

All computation was performed using Excel 2.2 on an Apple Macintosh SE, with the exception of the Marmur equation (12,13). This expression is not well suited to spreadsheet evaluation and so was calculated in BASIC on an Acorn A310 microcomputer for varying values of $\Phi_{\min}$.

Results

The relationship obtained between $\Phi_{\min}$ and W (Fig. 1) was in close agreement with that given by Marmur.

The refractive index of the soya oil was 1.473 ± 0.002, yielding a trial value of A_{11} (Eqns 8 and 9) of 5×10^{-20} J. The PCS mean diameter of the emulsion was 275 nm with a polydispersity of 0.11. The surface potentials at various electrolyte concentrations were calculated via Eqn 6 using the zeta potential data given previously (Washington, 1990). The total potential energy was then calculated on a grid of spacing 0.1 nm and the values of $\Phi_{\min}$ at each electrolyte concentration were found. The stability ratios were then read from the graph of Fig. 1. A_{121} was adjusted to minimise the residual error between calculated and measured stability ratios. This procedure is normal in colloid stability studies, primarily due to the difficulty in obtaining A_{121} from macroscopic data. It is feasible that an improved fit could be obtained by making small adjustments to the hydration parameters P_0 and λ, but this was not felt to be justified

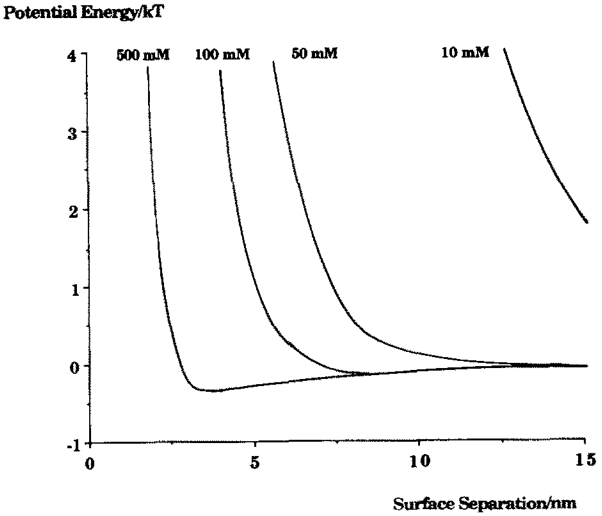

Fig. 2. Interdroplet potentials calculated for fat emulsions in sodium chloride solutions from 10 to 500 mM.

due to possible parameter redundancy, since all the parameters interact through Φ_{min}. The resulting optimised potentials are shown in Fig. 2. As expected, there was a barely detectable energy minimum at a sodium chloride concentration of 0.1 M, and a well-defined minimum at 0.5 M. Below a sodium chloride concentration of 0.1 M the potentials were completely repulsive.

The flocculation rate, expressed as stability ratio as a function of electrolyte concentration is shown in Fig. 3. As expected, a critical flocculation concentration was observed at a sodium chloride concentration of approx. 0.1 M. The calculated values are in good agreement at all but the lowest electrolyte concentrations.

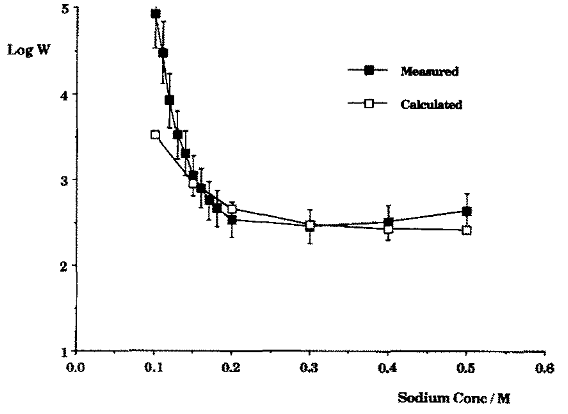

Fig. 3. Measured and calculated stability ratios of fat emulsions in sodium chloride solutions from 100 to 500 mM.

Discussion

The agreement between the measured and calculated stability ratios is good at high electrolyte concentration, and the prediction of the critical flocculation concentration is in accord with that observed. It should be stressed that the stability ratios are absolute values, and no scaling procedure has been used to adjust the relative magnitudes of the measured and calculated data. At lower electrolyte concentrations, the calculations severely underestimate the stability of the emulsion, by over an order of magnitude at a sodium concentration of 0.1 M. The reason for this is unclear but it is perhaps not surprising that the most difficulty should be encountered in this region, where the potential energy minimum is extremely shallow. At this point the potentials are a delicate balance of attractive and repulsive forces, and the shortcomings of the approximations of the separate contributions to the potential are apparent. However, it is noteworthy that an improved stability ratio could not be obtained at low electrolyte concentrations, even when unreasonable values were used for the Hamaker constant and hydration force parameters. This suggests that the Marmur model of flocculation may have some inadequacies for the description of this system.

Most previous approaches, from this laboratory and elsewhere, attempt to predict and manipulate the stability of fat emulsions and TPN mixtures by an understanding and control of the electrostatic repulsive potential, normally as measured by the zeta potential. As Fig. 2 shows, this factor holds the balance between the attractive and repulsive forces, and so this approach is fundamentally sound. However, the presence of the hydration potential, which has previously been neglected in this system, leads to interaction potentials which are fundamentally different to those predicted by DLVO theory, since they show no primary potential energy minimum. Consequently, this model predicts a reversible secondary flocculation at all monovalent electrolyte concentrations above the CFC, suggesting (as experience confirms) that the flocculated TPN system is inherently redispersible if it is diluted into a suitable medium which lowers the ionic strength suitably. In this respect, the behaviour of the system resembles closely that of phospholipid vesicles as described by Gamon et al. (1989).

This might suggest that flocculation in TPN mixtures is not of severe consequence. This is unfortunately not the case for a number of reasons. Firstly, the flocculated state may facilitate coalescence. The probability of droplet coalescence is almost certainly higher in a flocculated state due to the closer average spacing of droplets in the floc than in the dispersed emulsion. Secondly, the potential energy functions of the droplets in plasma components are unknown, so it cannot be predicted whether or not the system will deflocculate on administration. The adsorption of plasma components is likely to add a considerable complication to the interdroplet potentials, in the form of complex steric forces between the droplets. Finally, there is the wholly practical problem that, in a flocculated system, the administration of fat is likely to be irregular depending on the distribution of lipid within the bag.

The importance of the hydration potential leads to the suggestion that we may be able to significantly manipulate the stability of a TPN mixture by adjusting this parameter in addition to the electrostatic repulsion. Unfortunately, means by which this may be achieved cannot be predicted, largely due to the poor understanding of the structure of water near interfaces. It is possible, however, that we may already unwittingly be manipulating this parameter by the addition of amino acids and carbohydrates. The stabilizing effect of amino acids in particular is not well explained by their effect on the electrophoretic properties of the emulsion; most amino acids do not affect the zeta potential to a significant degree (Washington, unpublished observations) and so a detailed mechanism for their effect has previously been lacking. However, the hypothesis that amino acids increase the stability of the TPN mixture by increasing the hydration repulsion is feasible; since the amino acids are zwitterionic they may allow hydrogen-bonding forces to be transmitted further into the bulk from the interface, and hence could increase the decay length λ of the hydration repulsion. Alternatively, the presence of the amino acids at high concentration could lead to an additional solvent repulsion term analogous to that of water. Experimental tests of these hypotheses are currently being considered.

It should be stressed that the contribution of the hydration forces in the present model relies on the accurate value of the parameters measured by Lis et al. for phospholipid surfaces. If these are significantly in error for the present system, this will probably be manifest as a best fit for an incorrect value of A_{121} and hence A_{11}. Although the value of A_{11} is not known from other studies, it is encouraging that the value obtained from the colloid stability data is close to the trial value derived from the dispersion formula (4.75×10^{-20} J measured, 5.0×10^{-20} J calculated). The value of the Hamaker constant is also broadly in line with those obtained experimentally for other lipophilic materials, for example 7.13×10^{-20} J for phospholipids (Gamon et al., 1989) and 5.04×10^{-20} J for dodecane (Hunter, 1987). The effect of high concentrations of solutes such as electrolytes, amino acids, and carbohydrates on the Hamaker constant is almost completely unknown. It is desirable to eliminate A_{11} as an adjustable parameter and a calculated value will be used for future studies. The values provided by the dispersion theory of Lifshitz are considered to be at least as accurate as those obtained experimentally.

The stability ratio as calculated by the Marmur expression essentially describes the degree of flocculation as a dynamic equilibrium between flocculated and deflocculated droplets. The higher the stability ratio, the lower the fraction of flocculated material, and vice versa. This provides a qualitative explanation for the phenomenon often observed in TPN mixtures that only a small proportion of the droplets have formed a cream layer, while the remainder stay in suspension. In simple DLVO theory the system either has sufficient repulsion to stabilize it, or it is completely flocculated. It should be stressed that the ability of the current description to explain the partially flocculated state is a consequence of the reversible equilibrium nature of the flocculation and the absence of a potential energy barrier.

Although the calculations described here only treat a simplified model system at a superficial level, the agreement between theory and experiment is encouraging. It is possible that extensions of theoretical approaches such as this will provide a sound basis for the calculation of TPN mixture stability, ideally in the form of a piece of software usable by the pharmacist on a routine basis. This goal will require some considerable further effort but is being vigorously pursued.

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