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Invited Review

The stability of intravenous fat emulsions in total parenteral nutrition mixtures

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Summary

This article discusses methods for the study of the stability of fat emulsions in TPN mixtures, and their interpretation in the light of current knowledge of colloid stability. The emphasis is placed on methods of quantitatively calculating the stability of complex TPN mixtures; the methods currently under development by the author are described in detail.

Introduction

The essential fatty acid requirements of intravenously fed patients are commonly met by administering triglyceride oils which have been emulsified in water. This procedure has been used for a number of years; the modern commercial product is an emulsion with a mean droplet diameter of 200–300 nm, and is typified by 'Intralipid 20%' (Kabi-Vitrum, Sweden) and several similar materials.

The intravenously fed patient also requires a number of other nutrients, including amino-acids, electrolytes, carbohydrates, and trace elements. It is desirable to mix all of these materials, together with the emulsion, in a single container for rapid and efficient administration to the patient. Unfortunately early attempts to do this resulted in the rapid destabilization of the emulsion, manifest as the production of large oil droplets or the separation of cream or oil layers. Consequently it was for many years the practise to administer the emulsion from a separate container to the remaining nutrients, and manufacturers instructed that nothing was to be mixed with the fat emulsion. However, with experience it became evident that not all admixtures containing emulsions were unstable, and that some mixtures could be stored for days or even weeks without significant separation (Gove et al., 1979; Burnham et al., 1983; Du Plessis et al., 1987; Sayeed et al., 1987a,b). Equally other mixtures separated with great rapidity. Consequently, under pressure for more convenient administration regimens, emulsion manufacturers began to approve selected mixtures and to publish recommended regimens that could be premixed by the pharmacist in a single container, and stored for a short period before use. These became known as total parenteral nutrition mixtures or TPN mixtures.

The nature of the emulsion instability, its flocculation by electrolytes, has been understood in

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general terms for some time. A number of early studies highlighted the problems caused by the electrolytes and the protective effect of the amino-acids (Gray and Singleton, 1967; Black and Popovich, 1981; Whateley et al., 1984). However, due to the considerable complexity of the mixtures, many empirical studies of the stability of even a wide range of mixtures provided little additional knowledge, particularly since these were not interpreted in the light of modern colloid stability theory. It is only more recently that any real depth of understanding has been gained, largely due to the introduction of sophisticated instruments for the measurement of emulsion surface potentials and flocculation rates (Dawes and Groves, 1978; Kawilarang et al., 1980). However, despite the current level of knowledge, stability problems and unpredictable behaviour are still observed in parenteral nutrition mixtures. Consequently the pharmacist still needs to exercise caution in compounding, and it is hoped that an explanation of the physical basis of the emulsion stability will be of value in predicting and avoiding problems.

There are a wide range of additional stability problems which occur in TPN mixtures, particularly when drugs are added; these have been reviewed by Niemiec and Vanderveen (1984) and no attempt will be made to cover this field here.

Macroscopic Effects

A number of phenomena related to emulsion stability are observed in TPN mixtures and it will be useful here to describe these and to define precisely some terminology which is widely abused. The emulsion is an opaque white liquid; its individual droplets are effectively invisible on visual or microscopic inspection since they are smaller than the wavelength of visible light. The material is stable for several years, and retains its appearance in stable TPN mixtures, which are of uniform colour and turbidity with no visible oil droplets.

There are two separate processes which may lead to a change in the macroscopic appearance of the TPN mixture (Fig. 1). The droplets of emulsion may *coalesce* to form larger droplets, or they

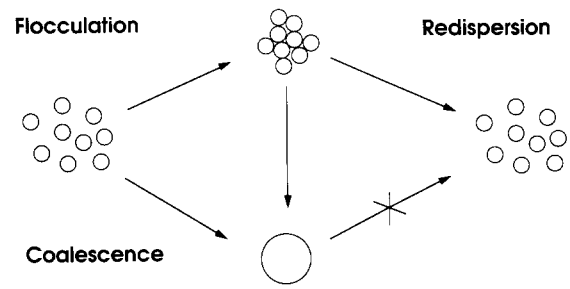

Fig. 1 The relationship between flocculation and coalescence in emulsions.

may *flocculate* to form aggregates of smaller droplets. Since the droplets in the flocs are relatively closely packed, flocculation may precede or accelerate coalescence to form larger droplets. Although the formation of flocs is well-understood, the coalescence process has been less widely examined.

Normally both flocculation and coalescence are manifest by the separation of the emulsion into separate regions of different appearance. If an upper layer of free coalesced oil is visible, the system is said to have cracked or broken, while the formation of an opaque white layer is termed creaming. The cream layer consists of droplets which have not undergone coalescence and consequently retain the original (~300 nm) droplet diameter, but are in an aggregated state. Both processes may occur simultaneously in a single mixture. The formation of a cream layer affords a highly misleading measure of emulsion stability. Separation of the layer is controlled by the time required for the aggregates to float to the top of the container, which is governed by Stokes' law. If the time taken for droplets and flocs of various sizes to rise 10 cm (a typical container dimension) in water is calculated, Stokes' law indicates that a 100 μm droplet will rise in about 3 min, a 10 μm droplet in about 5 h, and a 1 μm droplet in about 20 days. Microscopic examination suggests that flocs are of the order of 10–50 μm in size, and so would take several hours to rise to the top of the container. Thus, a creaming time of, e.g., 24 h suggests that the flocs are approx. 5 μm in size, but provides no information concerning their formation rate (which as we will see is considerably faster).

Studying TPN Mixture Stability

A wide range of techniques have been applied to the study of TPN mixture stability. The most popular techniques are particle sizing methods, but these often reveal only a part of the behaviour of the system. The fundamental problem with using particle size analysis to study TPN mixtures is that most sizing techniques demand a diluted emulsion sample. Dilution of the sample changes the environment of the emulsion and so may often lead to redispersal of any flocs present. Large coalesced ('secondary') droplets cannot be dispersed without applying considerable shear, and particle sizers will detect these successfully. Thus it is a general rule that only coalescence, and not flocculation, can be studied in most particle size analysers. The most straightforward method of studying flocculation is to examine the undiluted mixture in thin film under the microscope, when clumps of emulsion droplets, often tens or hundreds of microns in extent, become visible.

Particle sizing techniques — general considerations

There are a number of basic difficulties associated with the use of particle size analysis in the study of TPN mixtures. The first of these is due to the small volume of the sample examined by the instrument. Typically a Coulter counter may use a 100 μl TPN sample diluted into 100 ml, and then measure the distribution of droplets in 0.5 ml of this suspension, corresponding to 500 nl of the original TPN mixture, which is a fraction of 1.7×10^{-7} of a 3 l bag. Consequently, if the mixture contains only a few large droplets, the statistical probability of sampling them is remote; however, they can contain a significant amount of oil. For example, if the 500 nl sample contained only one 100 micron droplet, this would correspond to a mass of oil of 3 g in a 3 l bag. As the droplet size increases, the power of particle size analysis to detect a mass of oil decreases rapidly, solely due to statistical sampling considerations. For this reason visual inspection (possibly aided by Sudan red) is extremely useful.

In order to overcome the problems of sampling, a common suggestion is that the sample should be taken from the top of the container, which will have collected the larger droplets by sedimentation, thus increasing the probability of sampling them. This is a highly unsatisfactory idea, since the degree of concentration obtained by this means cannot be assessed, and moreover will be time-dependent as droplets of different sizes continue to accumulate near the top of the container. Consequently it is essential to mix the container gently but thoroughly before sampling.

This mixing introduces another potential error. If the mixture has creamed, mixing at the sampling intervals will redistribute the droplets. Assuming that the coalescence rate in the cream is different to that in the bulk (since the droplets are more closely packed) then the mixture will coalesce at a different rate than if it were undisturbed, and the true stability of the mixture will not be measured. Thus a rigorous study would make up a number of bags of similar composition and sample each one only once at a particular time. This is rarely performed; there is much psychological inertia against taking a 1 ml sample from a 3-litre bag and discarding the remainder!

Sampling is performed by withdrawing an aliquot with a syringe, then delivering a fraction of this sample into the appropriate instrument. Concern has been expressed that the shearing of the sample in the syringe needle may cause significant coalescence. Limited trials to assess the importance of syringe shear have been undertaken in the author's laboratories, but detected no change in size distributions by laser diffraction (Malvern Mastersizer) after withdrawing samples through needles from 14 to 26 gauge at varying rates. It would seem prudent, however, to sample carefully through a wide bore needle.

The application of some of the commonest particle size methods to the study of TPN stability will now be discussed. It will be helpful to refer to Fig. 2, which shows a typical particle size distribution for a fat emulsion (20% Intralipid). The distribution is approximately lognormal with a mean diameter of 280 nm.

Photon correlation spectroscopy (PCS)

This is a light scattering technique which uses fluctuations in scattered light intensity to measure the velocity of Brownian diffusion of the droplets,

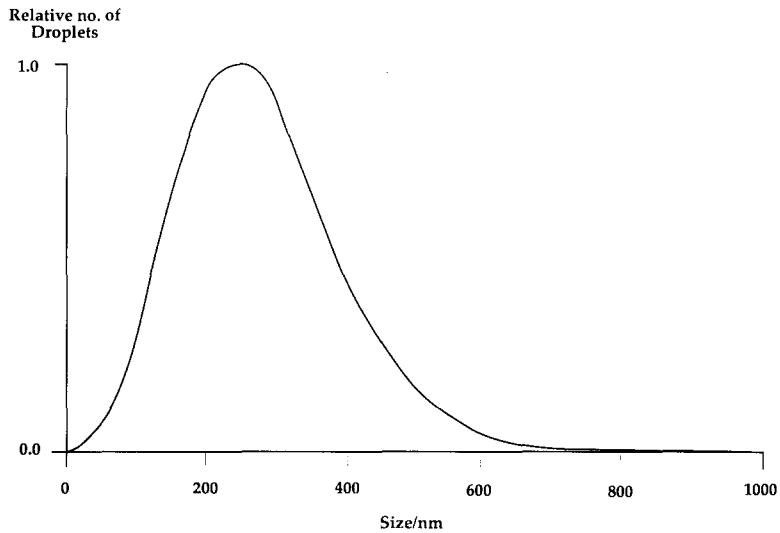

Fig. 2 Particle size distribution of Intralipid 20% (log normal function, PCS mean diameter 280 nm, polydispersity index 0.12).

and hence infers their diameter. It is sensitive to droplets of diameter 10 nm to 2 μm. Consequently it can detect the majority of the droplets in a fat emulsion or TPN mixture and so provides an accurate measure of the mean droplet diameter. The main problem with PCS is the algorithmic difficulty of extracting any information other than the mean diameter and a crude measure of the width ('polydispersity') of the distribution. For this reason it is difficult to detect the few large secondary droplets in the bulk of smaller droplets, and it is common to be unable to detect any change in the PCS mean diameter of a sample which shows considerable coalescence by other measurement methods. Consequently PCS is not the method of choice for the study of fat emulsion stability in TPN systems. The sample must be extremely dilute in order to prevent secondary light scattering, so flocculation would also not be detected by this technique.

Laser diffraction

This is a technique which is commonly confused with PCS, but the two are quite distinct. Laser diffraction measures the angular distribution of light scattered by the diluted sample (again flocculation is not detectable) and detects droplets from 0.5 nm to 200 μm. This makes it ideal for studying the accumulation of larger secondary droplets. It has the added advantage that it has poor sensitivity to the small uncoalesced oil droplets, which are largely below its lower detection diameter, and so only the largest droplets in the distribution are detected. Typical results (from a Malvern Mastersizer) are shown in Fig. 3. The size range can be changed by changing the focal length of a lens, but even with the smallest size range there is some underestimation of the proportion of droplets below 0.5 μm. The mean diameter measured by the instrument is thus larger than that measured by photon correlation, and so should be interpreted with caution. The main problem with the technique is that it provides only the shape of the particle size distribution, and does not give an absolute count of the number of large droplets. The instrument requests the operator to add the sample ad libitum until the desired scattering intensity is reached, and so the droplet count is unknown. It is possible to have two samples, one containing 10 times as many large droplets as the other, and obtain similar size distributions since the instrument has told the operator to put in 10 times as much of one sample as the other! This problem is even more severe when an unknown fraction of the smaller droplets in the distribution is below the lower detection diameter of the in-

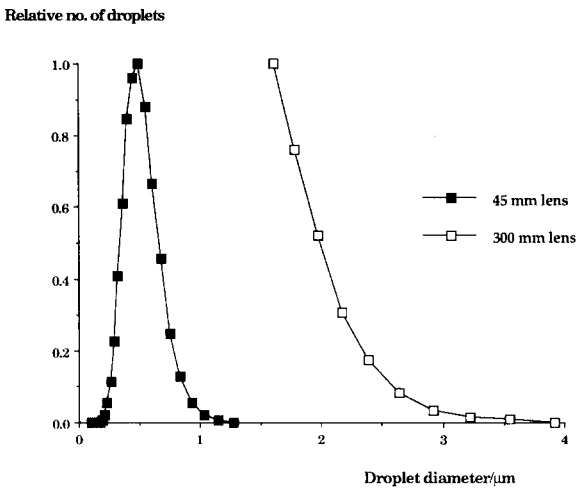

Fig. 3. Laser diffraction size analysis of Intralipid 20% using Malvern Mastersizer with 45 mm and 300 mm focal length lenses.

strument. Nevertheless, laser diffraction is still highly useful for the study of emulsion size distributions in TPN mixtures. As Fig. 3 shows, the detectability of droplets in the 1–5 μm range is extremely high when using the longer focal length lenses.

The Coulter counter

This familiar instrument works by flowing the sample through a pinhole and measuring the changes in electrical resistance induced as the droplets occlude the hole. Since only one droplet should pass through the hole at a time, the sample must be highly diluted, and again flocculation cannot be measured using this technique. A useful size range is that provided by a 30 μm pinhole, which measures the diameter of droplets in the size range 1.5–10 μm. The overwhelming advantage of this technique is that it provides an absolute droplet count within specified size limits, in contrast to the relative count provided by laser diffraction.

There is a subtle error associated with the measurement of droplet size distributions in fat emulsions by Coulter counter, which is not widely appreciated. As pointed out above, the sample must be dilute so that only a single droplet passes through the pinhole at any time. If two drops pass through together, the machine cannot distinguish them from a single large drop; the resultant error is termed a coincidence. Normally the Coulter counter provides an indication of the magnitude of the coincidence error as the sample is being prepared; it does this by calculation from the number of droplets that it counts, and parameters such as the pulse width and count rate. Unfortunately, when fat emulsions are studied, there is the possibility of a coincidence between a large droplet and one or more small (primary) droplets, which are below the lower size limit of the instrument. These cannot be allowed for in the instrument's estimate of coincidences, since the instrument cannot detect these smaller droplets; moreover, since there are vastly many more small droplets than large ones, the probability of these coincidences is extremely high. Although the submicron droplets themselves may be below the lower size limit for the pinhole in use, the presence of one or more of them in the pinhole when a larger droplet is passing through will lead to an increased size measurement for that droplet. Consequently fat emulsions must be sized only in very dilute suspensions; where the instrument manufacturers would recommend a coincidence level of 10% as being suitable for most work, the author would not exceed a coincidence level of 2% to avoid this particular error. The presence of this phenomenon can be detected by measuring the size distribution at a range of sample dilutions (this is an essential check of any particle size analysis protocol). At higher sample concentrations the particle size distribution will be shifted to larger sizes in the manner expected from coincidence errors; however this will happen at concentrations significantly lower than those suggested by the coincidence count provided by the instrument. The manufacturer's recommended coincidence levels for certain degrees of error have presumably been calculated assuming that the instrument can detect all the droplets in the sample, and may not be appropriate when a large number of droplets are below the lower detection diameter.

A further potential source of error arises from the need to dilute the emulsion into an electrolyte solution in order to measure the droplet size. The electrolyte solution used is 0.9% saline, which is

0.15 M, and as such will be above the critical flocculation concentration of the emulsion (q.v.). This will lead to a slow flocculation of the emulsion in the instrument. This source of error has not been examined in detail. The flocculation rate should be low since the sample is highly dilute, but it would seem sensible to measure diluted emulsion samples rapidly in order to prevent the formation of aggregates. There appears to be no reason why an ionic surfactant such as sodium lauryl sulphate should not be added to the electrolyte to prevent this problem.

Optical microscopy

Microscopy is one of the few methods which can be used to study flocculation in concentrated TPN samples. Microscopy demonstrates that flocculation occurs extremely rapidly, and certainly within a few min of compounding. Fig. 4 shows three images under low magnification of a simple TPN mixture in a thin film on a microscope slide. At $T=0$ the cover slip is sheared to disperse the flocs, and images captured over the next 1–2 min demonstrate that the flocs form within 1 min, and that flocculation is complete within 2 min. These flocs are quite large due to the nature of the particular TPN mixture studied here; smaller flocs would form even more rapidly. Consequently flocculation is complete as soon as the TPN mixture has been compounded; creaming would be observed hours later, as predicted by Stokes' law, but it would be false to conclude that the mixture was stable until the cream appeared.

Microscopy has a number of disadvantages for quantitative study, the most obvious being the difficulty of obtaining numerical information from the images. An early method of measuring flocculation rates microscopically, termed rheoscopy, was described by Klose et al. (1972) and Hassan (1982) and applied to the study of TPN mixtures by Hansrani (1980). The flocculating emulsion is placed between two slides and the light transmission measured using a microscope equipped with a transmitted light detector. Shearing of the sample between the slides causes deflocculation, and the flocs re-form over the next few min. The process can be followed by measuring the changes in light transmission. The method does not appear to have been widely used.

Microscopy is also widely used to examine emulsions for the presence of large droplets, on the assumption that the counting statistics are improved over those obtained for the small volumes examined by particle sizers. A hemocytometer should be used for counting, since it is necessary to know the sample volume; normally the emulsion will require dilution by a factor of about 10 in a 0.1 mm well; thus the volume of emulsion in a 1 cm^2 counting area is 10^{-3} cm^3, which is a factor of 10^3 greater than the amount measured in, e.g., a Coulter counter. This assumes that the whole slide area is examined; this is rarely done, but the counting statistics should still be superior to those provided by many particle size analysers. Manual counting is tedious and error-prone; as yet however there appear to have been no published accounts of the application of image analysis to the microscopic study of fat emulsions.

Electron microscopy

Electron microscopy is useful for particle size analysis of submicron particulates, but has a number of disadvantages for the study of fat emulsions. Considerable sample preparation is required, and the loss of part of the sample into the instrument vacuum cannot be discounted. The lipid layer around the emulsion can be stained with heavy metals such as osmium or uranium (Schoefl, 1968). Hamilton-Attwell et al. (1987) describe the method in detail.

Turbidimetry

The turbidity of an emulsion sample is a function of the droplet or floc diameter in the sample. Unfortunately a fat emulsion is so turbid that quantitative measurements can only be made in dilute solutions, with phase volumes of the order of 0.1% or less, or in thin films. The flocculation rate is then taken to be proportional to the rate of increase of turbidity. It is possible to calculate the absolute formation rates of multiplet flocs from light scattering theory (Lichtenbelt et al., 1974) but this only provides an approximate result due to the polydispersity of the sample.

The major area of application of turbidimetric measurements, used extensively in the author's la-

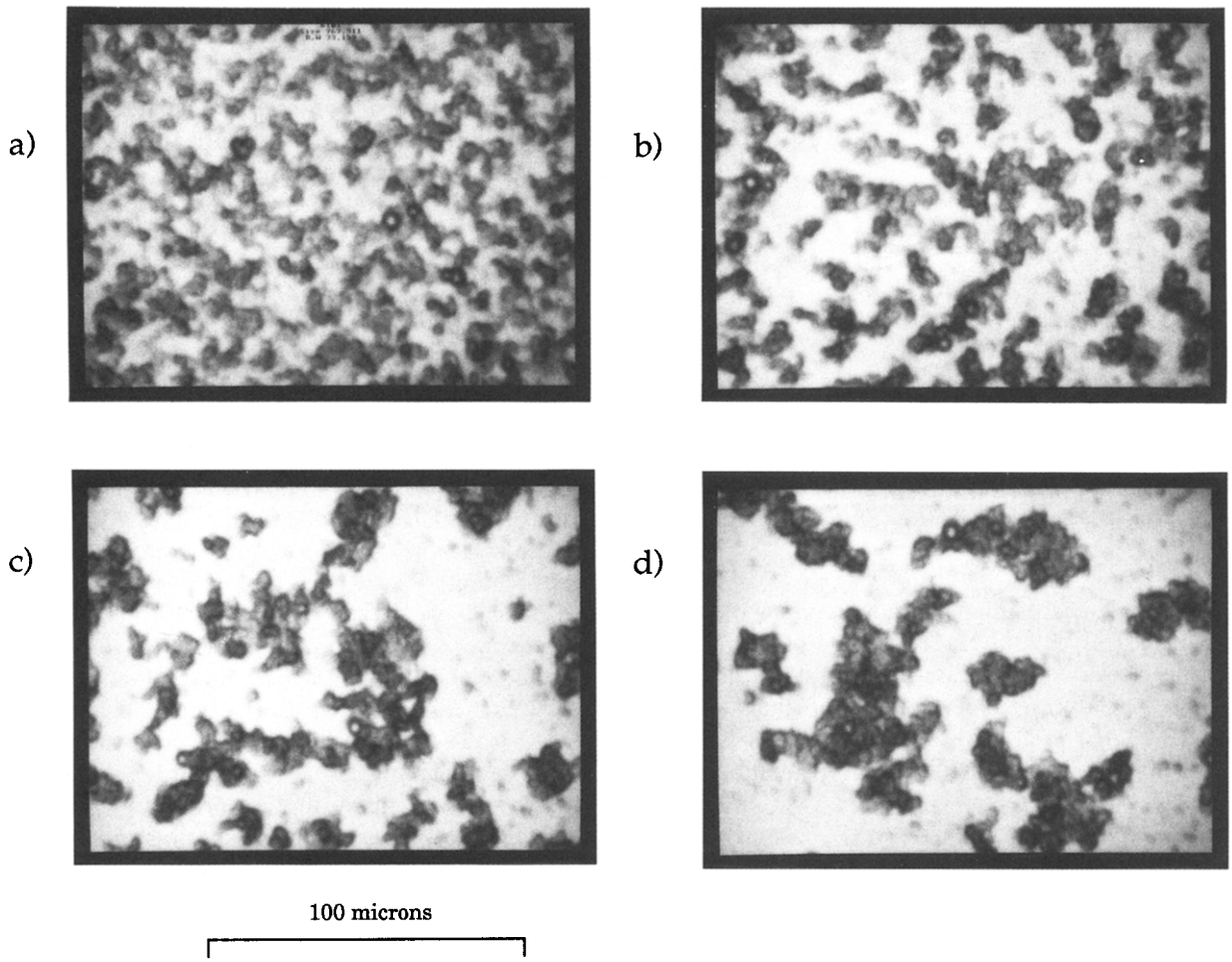

Fig. 4 Flocculation of Intralipid 20% in 2 mM calcium chloride; (a) t=0, (b) t=10 s, (c) t=20 s, (d) t=2 min.

boratories, is to study the effects of the continuous phase composition on the flocculation rate of a dilute emulsion sample (Washington and Davis, 1987; Washington et al., 1989, 1990; Washington, 1990a,b). This allows a systematic appraisal to be made of the effects of electrolytes, amino-acids, carbohydrates, and pH on emulsion stability. In such studies it is tacitly assumed that the flocculation of the dilute emulsion correlates with that in a similar concentrated TPN system in all but its absolute rate. This area has been little explored and the author would welcome more experiments to test this assumption critically. It is the author's experience that phenomena in simple electrolyte systems correlate qualitatively in both dilute and concentrated systems. Thus Fig. 5 shows the flocculation of dilute Intralipid 20% as a function of calcium concentration, as measured by turbidimetry, and the percentage of droplets larger than 1.2 μm in mixtures containing 10% w/v fat (i.e. 50% Intralipid 20%) by laser diffraction. This latter result was obtained using a thin-film cell which is currently under development for sizing of highly turbid (undiluted) samples. There is a reasonable correlation between these indicators of flocculation, but whether or not it extends to more complex systems is not wholly proven.

Zeta potential measurement

The ability to measure zeta potential is of con-

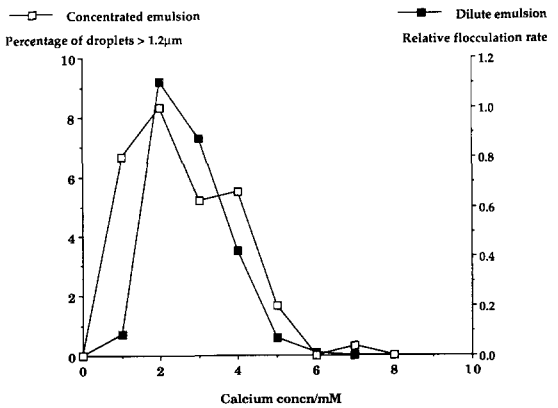

Fig. 5 Flocculation of Intralipid 20% by calcium by turbidimetry in dilute suspension and by laser diffraction of thin film of undiluted emulsion/calcium chloride.

siderable value in gaining an understanding of the stability of any colloidal system. Zeta potential is measured by electrophoresis; the emulsion droplets are placed in an electric field and their drift velocity is measured. The zeta potential can then be calculated from the Smoluchowski equation:

$$\zeta = \frac{u_e \eta}{\epsilon_0 D}$$

where ζ is the zeta potential, u_e the electrophoretic velocity, η the viscosity, D the dielectric constant of the suspending medium, and ϵ_0 the permittivity of free space.

Early instruments used direct microscopic observation of the droplets for the velocity measurement (e.g. in the Rank Microelectrophoresis instrument). This method was tedious and error-prone, and has been largely replaced by light scattering methods in which the droplet velocity is found from the doppler shift of light scattered from the moving droplets (Malvern Zetasizer series and Coulter Delsa). The methodology, and much useful theory, has been covered by Hunter (1981).

Both the manual and light scattering methods of electrophoresis require a dilute emulsion sample; the problem faced by the experimentalist is to dilute the emulsion or TPN mixture into a medium which accurately reflects the environment of the droplet in the system of interest. In the case of model studies in simple electrolyte solutions, this presents little difficulty; however it is much more difficult to measure the zeta potential of a droplet in a TPN mixture. It is not adequate to dilute the TPN mixture into water since the zeta potential then measured is that of the emulsion in water, and does not reflect the potential stabilizing the emulsion in the TPN mixture. The procedure used in the author's laboratories is to compound a copy of the TPN mixture for dilution purposes, omitting the emulsion component. It is necessary to add additional water to account for that contributed by aqueous phase of the emulsion (80% of the emulsion volume for 20% Intralipid). When the TPN mixture or emulsion is diluted into this medium and the zeta potential measured, two factors become evident. Firstly, the zeta potential is very low; typically within 1 or 2 mV of zero (the emulsion in water has a zeta potential of −40 to −50 mV) and can be positive or negative, depending on the particular TPN mixture studied. It is not easy to make accurate measurements of such a small potential, which is of the same order of magnitude as the accuracy of the instrument. Secondly, the dilution medium contains a great deal of electrolyte, and so carries a large current, which contributes to sample heating and electrolysis effects. These factors combine to make the measurements extremely difficult, and to date the author has not been successful in measuring zeta potentials in TPN mixtures with systematically varying composition.

Visual inspection

The problems of sampling from a large volume make visual methods the most reliable for the detection of large (~1 mm) droplets of floating oil. This is normally performed in a well lit cabinet in which the operator can carefully control the lighting angle in order to obtain the best visualization. In order to make free oil more easily visible, a hydrophobic dye such as sudan red can be added (Muhlebach et al., 1987). The solid dye is added to the emulsion, shaken, and allowed to stand for 5–10 min, when any oil droplets present will be clearly visible.

Accelerated stability testing methods

In order to study the stability of colloidal systems over a convenient timescale, accelerated stability tests are often used. A number of these have been described, such as shaking, freeze-thawing, or autoclaving (Hansrani, 1980; Burnham et al., 1983; Davis, 1983). These processes are intended to speed up flocculation or coalescence, while retaining the rank order of stability of a number of formulations. While they are useful in studying the stability of unmixed emulsions, their application to TPN mixtures is more difficult. The temperature sensitivity of TPN mixtures is a largely unexplored area, which makes the application of methods based on heating or freezing rather tenuous.

Emulsion Composition

The properties and behaviour of a fat emulsion are highly dependent on its composition. The disperse phase consists of vegetable triglycerides as refined soya oil BP, although other oils have been used, such as safflower oil. These oils are emulsified using lecithin, normally from eggs; the use of soya lecithin is being examined by several manufacturers. The stability of the emulsions is largely determined by the composition and properties of the lecithin, since this determines the surface chemistry.

There is a wide literature on the properties of lecithin-coated surfaces, mainly derived from studies of membrane models such as liposomes (see e.g. McLaughlin et al., 1978; Eisenberg et al., 1979; Lau et al., 1981; Altenbach and Seelig, 1984; Macdonald and Seelig, 1987). This literature can be extremely useful in understanding the properties of fat emulsions. Lecithin is a heterogeneous mixture of phospholipids; that used as an intravenous emulsifier is highly purified but still contains a broad spectrum of materials. The composition of a typical intravenous lecithin (Ovothin 180; Lucas Meyer) is shown in Table 1. The majority of the lipids (80–90%) are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). These lipids are uncharged at physiological pH. It also contains smaller (2–5%) quantities of acidic lipids, largely phosphatidylserine (PS) and phosphatidylglycerol (PG). These lipids are ionized at pH 7, and so confer a surface charge of approx. −40 to −50 mV on the emulsion droplets, which contributes to their stability. Any substance which interferes with this charge is likely to alter the emulsion stability.

TABLE 1

Composition of a typical intravenous grade lecithin (Ovothin 180)

Phosphatidylcholine	approx. 80%
Lysophosphatidylcholine	max. 3%
Phosphatidylethanolamine	approx. 8%
Water	max. 2%
Triglycerides	max. 1%
Cholesterol	max. 2%
Peroxide value	max. 5
Acid value	max. 12
Iodine value	60–65
Phosphorus	3.5%
Nitrogen	1.7–2.0%

Colloid Stability

In order to understand the basis of the stability of fat emulsions in TPN mixtures it is helpful to understand some basic colloid stability theory. Colloidal particles either attract or repel one another under the influence of an interparticle force which varies depending on the separation of the particles and the interparticle environment. There are three components to this force:

Electrostatic. The particles carry a like charge, due to ionization of surface lipids, and so repel one another. The magnitude of this force is influenced in a complex manner by electrolytes. The charges between the particles are screened by ions in solution, and so the range of the forces decreases as ionic strength increases. The variation of force with distance is complex and several approximate forms are available; one of the most useful is due to Verwey and Overbeek (1948) and is applicable at high κa and low surface potential:

$$V_R = 2\pi\epsilon_0\epsilon_r a\Psi_s^2 \ln(1+\exp(-\kappa H)) \quad (1)$$

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 Stern potential is the potential at the mid-point of the layer of tightly adsorbed counterions; in practise this is difficult to estimate, and is usually approximated by the zeta potential, which is the potential at the surface of hydrodynamic shear, and so is the potential measured by electrophoresis.

This force decays approximately exponentially with distance. The parameter κ is the Debye-Hückel constant, given by:

$$\kappa = 3.288\sqrt{I}\ (\mathrm{nm}^{-1})$$

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

Attractive or Van der Waals forces. These forces have their origin in the exchange of virtual photons between the droplets, and although their theory is complex (see e.g. Hunter, 1987) their form is reasonably simple. The most well-known approximate form for the force is:

$$V_A = \frac{A_{121}a}{12H}$$

While an alternative form (Schenkel and Kitchener, 1960):

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

allows empirically for the effects of retardation through the characteristic oscillator wavelength l. In practice retardation is not of major importance at small particle separations. H is the particle surface separation, and a the particle radius. The constant A_{121} is the Hamaker constant of soya oil (medium 1) in water (medium 2). The Hamaker constant is characteristic of the disperse material *and* the medium in which it is immersed. Explicitly, if a particle with Hamaker constant A_{11} is immersed in a medium with Hamaker constant A_{22}, the interparticle force is determined by the effective Hamaker constant A_{121}, given by:

$$A_{121} = ((A_{11})^{0.5} - (A_{22})^{0.5})^2$$

Since the Hamaker constant is dependent on the composition of the continuous phase, it is possible that alterations in the composition (such as the addition of TPN components) may decrease or increase the attractive force between the droplets. Unfortunately the theory of the attractive force in multicomponent solutions is ill developed, and so the likely changes in the interparticle attractive force cannot be predicted. This is unfortunate, since, as studies of the effects of glucose demonstrate, changes in the magnitude of this force may be of some importance.

Hydration forces. These forces are due to the perturbation of the solvent structure as the droplets approach, and the structuring of the solvent by adsorption to the droplet, and so are always repulsive. They can be described by the empirical equation:

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

For phospholipid surfaces in water, the parameters P_0 and λ have been determined as 5.75×10^9 N m^{-2} and 2.6 Å, respectively, by elegant experiments on liposomes (Lis et al., 1982); currently similar values must be assumed for emulsions, despite the fact that the phospholipid monolayer around the emulsion may be structured differently to a phospholipid bilayer. These force constants may also vary with continuous phase composition due to solvent restructuring by ions, and adsorption of materials such as glucose and amino-acids to the droplet surface. At present there are no estimates of the importance of these continuous-phase effects. Hydration forces have little effect at long range but become extremely important at short distances. In the past they have often been neglected, but they radically influence the behaviour of the system.

The total potential of the system is found by addition of these components:

$$V_T = V_R + V_A + V_H$$

In the simplest model, at constant temperature the hydration and attractive forces are considered to be constant and the electrostatic force is the only variable. This is largely since it is the only component of the potential whose theory is well developed. The Hamaker constant is treated as a variable parameter or is derived by matching calculated and predicted stability data. This has been performed by Washington (1990b) and the resultant Hamaker constant of soya oil in water (A_{121}) was found to have a value of 6.5×10^{-22} J.

It is desired to calculate directly the stability of the emulsion in a particular environment. This is done by calculating the interdroplet potential from the above description. The stability of the emulsion can be calculated from the potential energy function by two alternative methods. That described by Fuchs (1934) assumes that the particle diffuses over a potential energy barrier into a deep potential well and that this process is not reversible. The model developed by Marmur (1979) models the kinetic equilibrium between the free droplets and those bound in the potential energy minimum corresponding to the flocculated state. The model is mathematically complex but ultimately describes the stability ratio as a function of potential energy well depth (Fig. 6) which may be found from the interdroplet potential calculations.

The emulsion stability is quantified as the stability ratio W, which is the ratio of the observed flocculation rate k_{11} to the diffusion-controlled rate k_D predicted by the Smoluchowski (1917) model:

$$W = \frac{k_D}{k_{11}}$$

where

$$k_D = \frac{8kT}{3\eta}$$

η is the viscosity of the medium, T the absolute temperature, and k Boltzmann's constant.

A low stability ratio (1–1000) thus implies an unstable or rapidly associating system, while a high stability ratio ($>10^6$) is characteristic of a stable system. Fig. 6 demonstrates, as expected, that a system with a deep potential energy well is unstable in the disperse state (low W), while one with a shallow well is unstable in the flocculated state (high W).

Effect of Electrolytes

Electrolytes were recognised as the source of most of the stability problems in TPN mixtures in

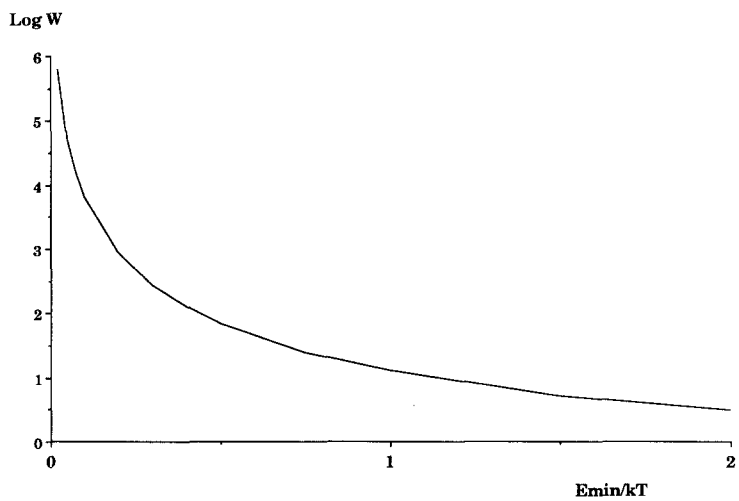

Fig. 6 Stability ratio as a function of potential energy well depth (from Marmur, 1979).

early studies (Gray and Singleton, 1967). Dawes and Groves (1978) found that the positive cations adsorb to the negatively charged emulsion droplet, and in doing so reduced its surface charge. This study detected many of the effects which are now familiar, such as the pronounced reversal of charge by calcium, and the similarity of behaviour between the monovalent electrolytes.

Neutralization of droplet charge by electrolytes allows the attractive Van der Waals force to predominate and hence the emulsion flocculates. The mechanism of adsorption of the ions is important in determining the detailed behaviour in the system, and we can distinguish two limiting cases of adsorption:

Nonspecific adsorption

This aspect of colloid-electrolyte interaction is well-known as the DLVO theory (Deryaguin, 1940; Verwey and Overbeek, 1948). In this case the ions are bound to the droplet only by electrical double-layer interactions with the charged surface. As the electrolyte concentration is increased, more ions adsorb, and at high electrolyte concentration the droplet zeta potential approaches zero. Since the only force causing adsorption is electrostatic, no further ion adsorption can occur when the charge is zero, so a charge of the opposite sign cannot be developed. A typical form for this behaviour is shown in Fig. 7. The droplet charge asymptotes to zero as the electrolyte concentration is increased.

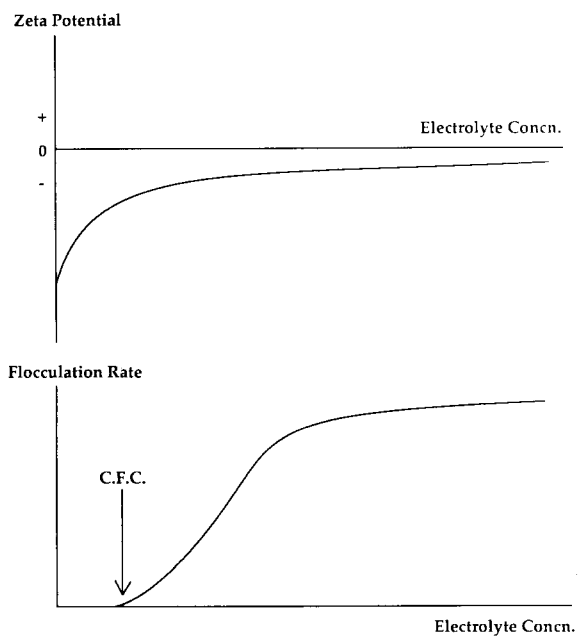

Fig. 7 Zeta potential and flocculation rate of Intralipid 20% in the presence of a nonspecifically adsorbing electrolyte.

As the repulsive force due to the droplet charge decreases, a point is found where the attractive Van der Waals force is equal to the repulsive electrostatic force. At this point the emulsion begins to flocculate (Fig. 7). This point is called the critical flocculation concentration or CFC, and its value is given in the simple DLVO theory by:

$$\mathrm{CFC\,(mol\,dm^{-3})} = 87.4 \times 10^{-40}/z^6 A_{121}{}^2$$

at 25°C in water (Hunter, 1981); z is the charge on the flocculating cation.

As the electrolyte concentration is increased above the CFC the force between the droplets becomes more attractive, and the colloid flocculates more rapidly. Unfortunately the DLVO theory only provides the value of the CFC and does not allow us to calculate the actual flocculation rate; for this purpose the theory of Marmur, discussed above, must be used.

DLVO theory predicts that the CFC should depend inversely on the sixth power of the ion charge, and so the CFCs for monovalent, divalent, and trivalent ions should be in the ratio 1:1/64:1/721, or 1:0.016:0.0014. This is called the Schultze-Hardy rule, and is fairly well obeyed in practise. However, it has been widely abused, particularly in the study of TPN stability. We should note:

(a) The Schultze-Hardy rule applies only to nonspecifically adsorbing electrolytes, and cannot be applied to specifically adsorbing electrolytes (q.v.).

(b) The rule applies only to individual electrolytes, and it is not possible to 'add up the Schultze-Hardy contributions' in an electrolyte mixture to obtain a stability index. This is largely due to the fact that separate ions in a mixture compete for surface binding, rather than influencing the stability additively. It is now fairly widely accepted that calculations of TPN stability based on

Schultze-Hardy considerations do not correlate well with experimental data.

Specific adsorption

An electrolyte is said to be specifically adsorbed when, in addition to the electrostatic forces causing its adsorption to the droplet surface, it is bound or complexed by a specific chemical interaction. The droplet can then adsorb by complexation more ion than is required to neutralize its charge; consequently the surface charge passes through zero and ultimately acquires a sign opposite to that observed at low electrolyte concentration. This effect is referred to as charge reversal. The zeta potential typically varies with electrolyte concentration as shown in Fig. 8. As the electrolyte concentration is increased, a point is found at which the electrostatic repulsion becomes weaker than the Van der Waals attraction, and so flocculation occurs at a well-defined CFC. Increasing the electrolyte concentration causes the droplet charge to pass through zero; this is referred to as the point of zero charge or PZC. At this point the flocculation rate is a maximum; a further increase in electrolyte concentration causes a charge of the opposite sign to develop; since all the droplets have the same charge, the repulsive component of the potential increases and the flocculation rate decreases from its maximum value.

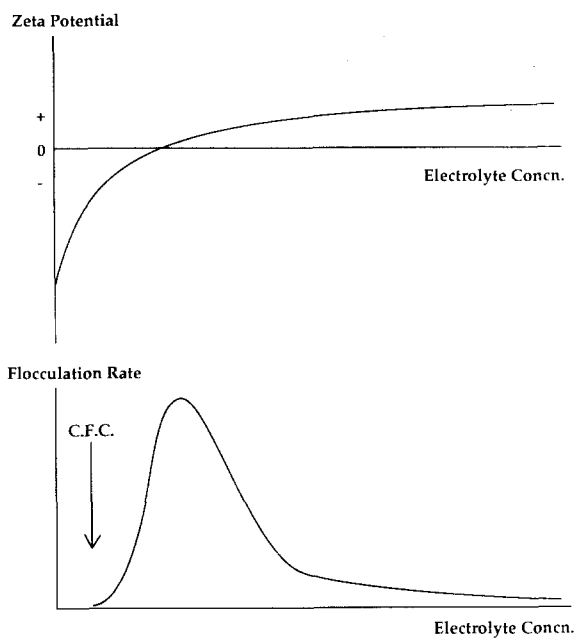

Fig. 8 Zeta potential and flocculation rate of Intralipid 20% in the presence of a specifically adsorbing electrolyte.

It should be noted that the exact form of the surface potential vs electrolyte function is not predictable by the Gouy-Chapman theory and the CFC will not be accurately predicted by the Schultze-Hardy rule. The surface potential depends on the form of the ion adsorption isotherm onto the surface. In practise Macdonald and Seelig (1987) found that several common forms for the adsorption isotherm led to similar dependencies of the zeta potential on electrolyte concentration, except at extremely high electrolyte concentrations.

It is found that fat emulsions adsorb monovalent ions such as sodium and potassium non-specifically, while divalent ions (calcium and magnesium) are adsorbed specifically. This is due to their complexation by the negatively charged acidic phospholipids such as PG and PS. Studies on liposomal phospholipid preparations have indicated that divalent ions also adsorb weakly to neutral lipids such as PC and PE, (Altenbach and Seelig, 1984), but this interaction is usually ignored in studying TPN mixtures. This adsorption can however be demonstrated in fat emulsions which are stabilized by pure PC with no charged lipids present (Washington et al., 1989), which become charge reversed at low calcium concentration, implying specific adsorption. A similar weak specific adsorption has been demonstrated for monovalent ions in liposomal systems (Eisenberg et al., 1979). Trivalent electrolytes cause strong charge-reversal, but are rarely present in sufficient quantity to demonstrate an observable effect.

It should be noted that the concentration of electrolyte which drives adsorption is the free solution concentration; thus in concentrated emulsion suspensions, more electrolyte will be required to cause charge reversal than that measured in infinitely dilute suspension. This is since a certain quantity of ions are required to 'fill up' the sites. Thus, for example, in extremely dilute emulsion solutions, the ferric ion at 0.1 mM charge reverses the droplets to +50 mV (Hansrani, 1980). If, how-

ever, the same 0.1 mM of ferric ion were added to a TPN mixture, much of the ion would adsorb to the emulsion surfaces, and the aqueous phase concentration would then be much lower; consequently the zeta potential at equilibrium would be much closer to its original value. In concentrated TPN systems it may be necessary to add several millimolar ferric ion to fill up the emulsion surface sites before a continuous-phase concentration of 0.1 mM is reached.

At present a detailed theoretical description of emulsion stability has been developed only for the case of emulsion plus nonspecifically adsorbing electrolyte (Washington, 1990b). If this is performed using the methods outlined above, the following conclusions are obtained. The interdroplet potentials, which are shown in Fig. 9 for selected electrolyte concentrations, are completely repulsive at electrolyte concentrations below the CFC of sodium (0.1 M) and higher sodium concentrations result in a weak potential energy minimum. Since there is no energy barrier, this is referred to as a secondary minimum (there is no primary minimum due to the powerful hydration forces at smaller distances). The depth of the minimum is of similar magnitude to the thermal energy available to the droplets (kT), implying that flocculation is a reversible process, and that an equilibrium exists between flocculated droplets in the minimum, and unflocculated droplets outside it. Since the minimum vanishes at lower electrolyte concentrations, the sample is deflocculated reversibly on dilution. The stability predicted from a combination of these potential energy calculations and the Marmur stability model agrees reasonably well with that measured experimentally, at all but the lowest electrolyte concentrations, where the stability of the emulsion is underestimated (Fig. 10). The Marmur theory of flocculation is particularly interesting for our purposes since it provides a partial explanation for the often-observed phenomenon of weak creaming or partial flocculation. Classical DLVO theory only allows a colloid to be in a flocculated or unflocculated state, and predicts that an emulsion is either stable or fully creamed, i.e. present as a cream phase with a clear subnatant. In practise intermediate stages are usually observed, in which a layer of cream of variable thickness is formed on top of the mixture, and a large fraction of the droplets remain unflocculated in the bulk mixture. Measurements of the thickness of this cream layer are often used as a crude measure of mixture stability. The Marmur model predicts a dynamic equilibrium between flocculated and unflocculated droplets, and thus naturally allows for the possibility of fractional flocculation. At this point an obvious question is 'when the fraction of flocs have floated to the top and removed themselves from the local system, why does further flocculation not take place in the bulk mixture to

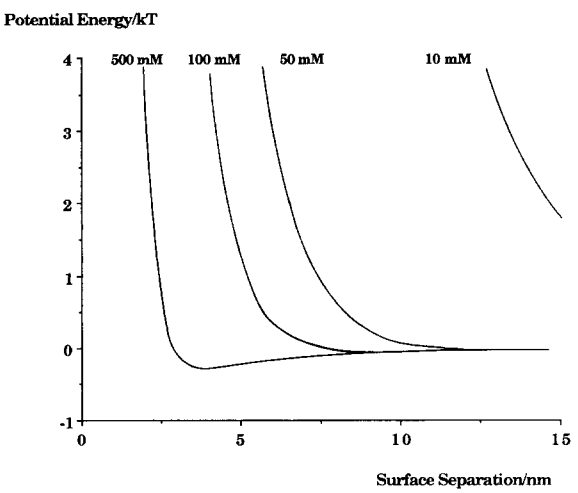

Fig. 9 Potential functions of Intralipid 20% in solutions of sodium chloride. Note the weak energy minimum in the more concentrated electrolyte solutions.

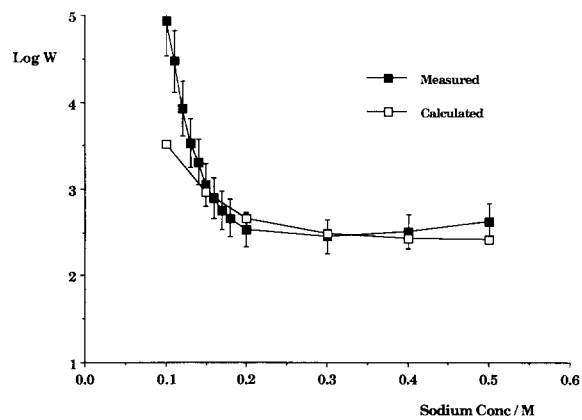

Fig. 10. Experimental and theoretically calculated stability ratios for Intralipid 20% in sodium chloride.

restore the equilibrium?' The answer to this is not yet clear, but it may be connected with a depletion of ions in the lower phase as they are carried to the upper phase, thus changing the local ion concentrations in the mixture and the cream layers.

Potential energy calculations in the presence of divalent (specifically binding) cations such as calcium have not yet been performed, and so the effects of these species must be discussed at a more superficial level. The zeta potential of a fat emulsion as a function of calcium concentration is shown in Fig. 11a, and the corresponding flocculation profile (by turbidimetry in dilute solutions) in Fig. 11b. A CFC is seen at 2 mM, a PZC at 3–4 mM, and an upper CFC at 5–6 mM. This behaviour is consistent with calcium being specifically adsorbed to the phospholipid surface.

TPN mixtures contain a range of ions, and so it is necessary to understand how the zeta potential and stability depend on solution composition when more than one electrolyte is present. As discussed above, contributions to surface charge from multiple ions are not additive, largely since the ions must compete for binding surface. Recently the author produced a simple empirical model for this behaviour (Washington, 1990a) in which the ions compete for binding sites on the emulsion surface; the mathematics generated was similar to that which has been used to describe competitive receptor binding. Although the description of the electrokinetics in this model was empirical, it predicted with good accuracy the variation of zeta potential in a mixture of monovalent and divalent electrolytes. In particular, competition between the two ions was evident; the presence of sodium caused the PZC produced by calcium to be shifted to higher calcium concentrations than in the absence of sodium. This competitive behaviour has now been confirmed by measuring the flocculation of Intralipid in the presence of a mixture of sodium and calcium chlorides (Athersuch et al., 1990b) (Fig. 12). As sodium is added to the mixture, the CFC and PZC move to higher divalent ion concentrations, and flocculation occurs over a wider range of divalent ion concentrations. It is possible that, once the effects of amino-acids and glucose are more fully understood, a set of curves of this type could form the

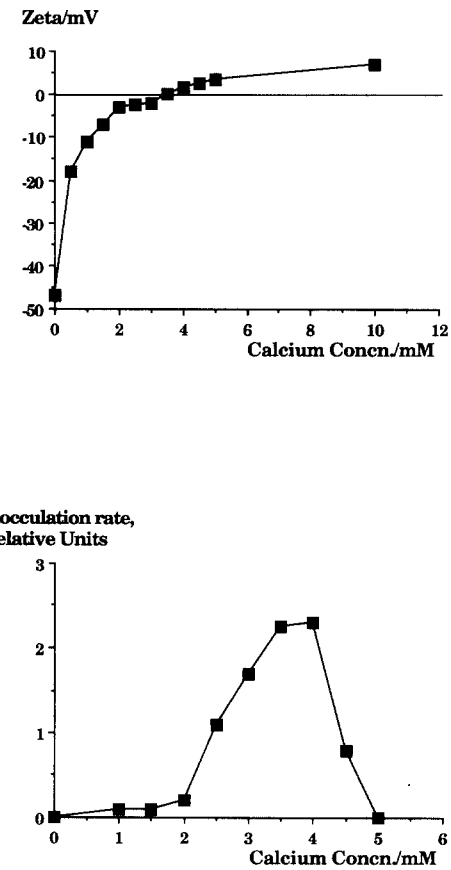

Fig. 11 Flocculation of Intralipid 20% in calcium chloride solutions (a) zeta potential, (b) flocculation rate.

basis for the prediction of TPN mixture stability.

The effects of anions in the system are usually ignored; Dawes and Groves (1978) found anions to be unimportant, which is not surprising, since they are repelled from the negatively charged emulsion droplet. There are, however, two cases in which the anion might have a significant effect on emulsion stability:

(1) The anion may chemically adsorb to the droplet surface despite the electrical repulsion. For example, sodium pyrophosphate appears to make the zeta potential of fat emulsions more negative in this manner (Dawes and Groves, 1978).

(2) If the emulsion is charge-reversed in the TPN mixture, anions will adsorb to it. For this reason it seems prudent to avoid divalent and triva-

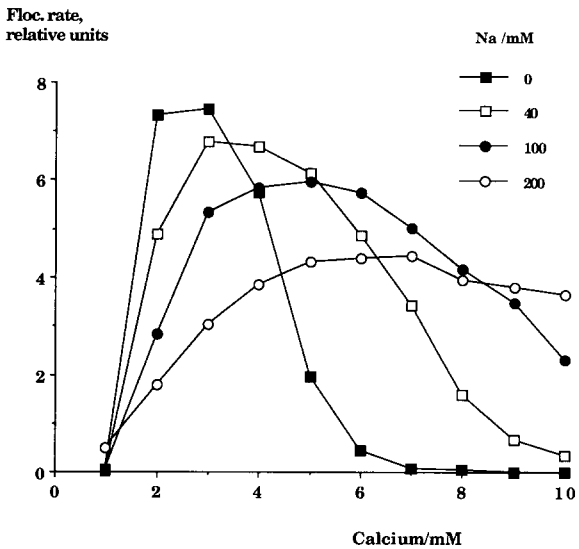

Fig. 12 Flocculation of Intralipid 20% in solutions containing mixtures of sodium and potassium chlorides.

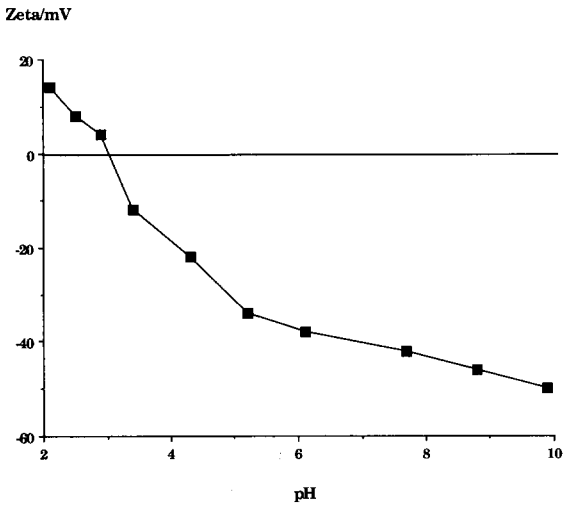

Fig. 13 Zeta potential of Intralipid as a function of pH.

lent anions in high electrolyte mixtures. The flocculating effect of heparin (below) is an extreme example of this effect.

Effect of pH

The effect of pH on surface charge is only a special case of electrolyte adsorption, since H^+ alters the droplet charge by specific adsorption in the same way as several other ions. H^+ is a special case since it is the ion gained or lost when the phospholipid ionizes; as such it is referred to as the potential-determining ion or PDI in the system. The basis for the discussion of pH effects is the pH-ionization profile of the phospholipid, which gives rise to a pH-mobility profile for the emulsion. A typical profile for Intralipid 20% is shown in Fig. 13. At pH 7 the ionization of the PS and PG induces a charge of -30 to -50 mV, and as the pH is reduced, this ionization is suppressed, until the charge is zero at a pH of 3.2. Further reductions in pH cause the protonation of the phospholipid and a positive charge results.

The effect of pH on the flocculation of the emulsion by another specifically adsorbing ion (Ca^{2+}) is shown in Fig. 14. It is not yet possible to calculate the exact form of this curve, but a qualitative explanation of the major features is straightforward. The profile at pH 7 is similar to that measured previously; if the pH is reduced below 7, then the zeta potential in the absence of calcium falls, and so less calcium is required to reach the CFC and PZC. This results in the profiles being shifted to lower calcium concentrations. Increasing the pH above 7 causes the zeta potential to increase, and the profiles are then shifted to higher calcium concentrations.

TPN mixtures have a pH of between 5 and 7 (Barat, 1987), due to buffering by the amino-acid content. The glucose component is acidic (as low as pH 3) due to decomposition during autoclaving, but does not normally influence TPN mixture pH due to the buffering effect of the amino acids. However, it is possible that a small change in pH during storage may lead to a significant change in stability if the mixture is close to a critical flocculation concentration.

Effect of Glucose

Glucose has often been considered to be an inert component of TPN mixtures, or to destabilize the mixture by virtue of the change in pH which it may induce (Black and Popovich, 1981). Auto-

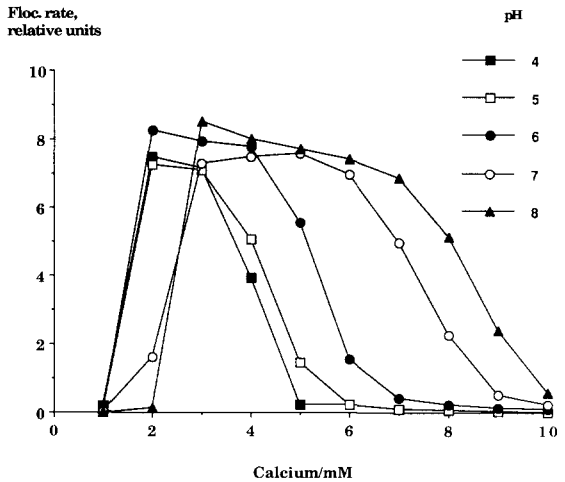

Fig. 14 Flocculation of Intralipid 20% as a function of calcium concentration and pH.

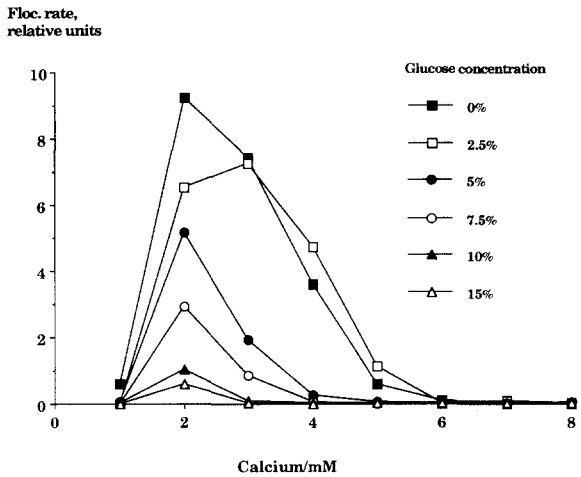

Fig. 15. Flocculation of Intralipid 20% as by calcium in the presence of glucose solutions.

claving of glucose solutions may cause the pH to fall as low as 3.0; at this pH the fat emulsion has very little surface charge. Since the amino acid component of the TPN mixture buffers the pH within the range 6.5–7.5, glucose is not normally observed to alter TPN mixture pH significantly.

Recent work suggests that glucose may have an important role in stabilizing TPN mixtures (Athersuch et al., 1990a; Washington et al., 1990). Addition of glucose was found to reduce the flocculation rate of emulsions in monovalent and divalent electrolyte solutions in dilute turbidimetric measurements (Fig. 15). The glucose did not affect the zeta potential, nor did it influence the position of the PZC or CFCs in the system. Consequently it could not be influencing emulsion stability via the electrostatic part of the interdroplet potential, and so must have been altering either the Van der Waals force or the hydration force. Although it was not possible to determine which force was being influenced by glucose, it was possible to calculate the likely magnitude of the changes in the forces, either as a change in the Hamaker constant of the continuous phase, or a change in the range of the hydration force. Further experiments are planned to distinguish between changes in these two force components.

Effect of Amino Acids

Amino acids are well-known to stabilize TPN mixtures against the flocculating effects of electrolytes, but the mechanisms involved are not well understood and several separate processes may be occurring. It is possible that the complexation of calcium or magnesium by certain amino acids may reduce their activity in solution. Surprisingly, amino-acids have little effect on the zeta potential, and the minor electrokinetic effects observed do not satisfactorily explain their behaviour. Recently the author has proposed that the effect of amino acids may be due to their influencing the hydration forces or solvent structure around the droplet, or to influencing the Van der Waals forces between the droplets, in a similar manner to that proposed for glucose.

A complete consideration of the effect of amino acid solutions may have to take into account the concentration of each individual acid, a formidable task. It is possible that some simplification can be made by considering in groups the acidic, neutral, and basic amino acids. Hardy (1989) has shown initial data which suggests that the ratio of basic to acidic amino acids should be greater than 1.5:1 for improved stability, but it is unclear to what extent this is coupled to small pH fluctuations. A range of commercial amino-acid solutions

were studied by Barat (1987), who found only a small variation in mixture stability using these products.

A number of workers have investigated the use of derivatives of amino acids, such as acetylarginine and acetyllysine. These do not appear to destabilize emulsions directly, but more work is required before their effects are understood. (Ishii et al., 1988).

Other TPN Components

Several other TPN components may influence mixture stability. The best known example is heparin, which causes massive flocculation at very low concentrations in mixtures in which calcium is present (Rattenbury et al., 1988; Rauff et al., 1988). This flocculation is only seen when the emulsion droplet charge is positive, and the most likely mechanism is that the negatively charged polyelectrolyte heparin is acting as a bridging flocculant and binding the positive emulsion droplets together (Johnson et al., 1989, 1990). The use of medium -chain heparins has been suggested as a possible solution to this problem.

There has been some discussion that trace mineral and vitamin additives such as multibionta can alter the stability of mixtures, and that this may be due to the presence of small amounts of solubilizing surfactants such as the polysorbates in these mixtures. At present this area has not been well explored.

Product Variability

Intravenous fat emulsions are based on materials from biological sources which contain many components, and whose composition may vary. Although much of this variability is removed by careful purification, lecithin in particular is a highly variable material. Since the stability of the emulsion is largely dependent on the level of acidic phospholipids, which are minor impurities in the lecithin, it might be expected that the product was variable in its surface charge, droplet size, and stability, and also that different batches of emulsion would make TPN mixtures with different stability characteristics. Some concern about this latter aspect has been expressed among compounding pharmacists for some time.

Despite the apparent variability of the feedstocks, the majority of commercial fat emulsions are well-known to display a remarkably small degree of variation in their properties. A more serious aspect of variability in TPN mixtures is the variability of the mixture composition. This is mainly due to variations in fill volume practises among manufacturers. Volume measurements of a range of parenteral products from several manufacturers demonstrated that some products contained exactly the stated volume, while others contained a 5–10% excess, and this, together with compounding variations, leads to small changes in mixture composition on a day to day basis. Although these variations are too small to be of nutritional significance, they may induce a large variation in the flocculation behaviour, particularly if the mixture is close to a critical flocculation concentration. Small fluctuations in pH may have a similar large effect on the flocculation state of the emulsion. Variations of this type will lead to unpredictable stability in a subgroup of TPN mixtures which are close to a critical flocculation concentration, so that these mixtures may be perfectly stable on one occasion but apparently unstable on another.

Clinical and Compounding Aspects

The author has encountered a wide variety of compounding practices in his discussions with pharmacists, and a number of general guidelines have become evident. The emulsion is at its least stable when mixed directly with those materials which alter its zeta potential, i.e. electrolytes or unbuffered glucose. Since amino acids have a protective effect against electrolyte flocculation and a buffering action, they should be added to the mixture prior to the emulsion. Many pharmacists have adopted the procedure of adding the emulsion last, which appears to be a good method since it ensures that the electrolytes are maximally diluted before emulsion is added. It is possible that the

components may stratify in the bag, so the contents should be thoroughly mixed prior to adding the emulsion. Since the emulsion appears to be unaffected by reasonable shear levels, vacuum filling, with its higher shear rate, does not seem to pose any problems. Initial experiments in the author's laboratory have detected no significant differences in mixture stability between different bag materials, although further experiments are required in this area.

Probably one of the most widely discussed questions arising from TPN stability concerns the safety of administering flocculated emulsions to patients. At present it is not possible to present experimental evidence to show either that flocculated mixtures are harmful, or that they deflocculate on administration. However, potential energy calculations (Washington, 1990b) have demonstrated that flocculation is weak and occurs in a secondary energy minimum. This implies that the emulsion will deflocculate reversibly if it is diluted into a medium in which it has a high zeta potential. Initial attempts to measure zeta potential of fat emulsions in plasma have suggested a rather variable value of -8 to -15 mV. It is not at present possible to determine if this leads to flocculation or deflocculation due to the complexities of the mixture. Plasma contains a range of proteins which may act as protective colloids, as well as approx. 2 mM calcium and 1 mM magnesium, part of which are protein-bound. Consequently the flocculation of emulsions in plasma will depend not only on the electrolyte concentration, but on the amount of ion-binding protein present. This is likely to lead to a range of behaviour in vivo; an indication of this has been given by LeVeen (1961, 1965) who divided subjects into 'creamers' and 'non-creamers' depending on the ability of their plasma samples to flocculate added emulsion.

In order to avoid flocculation, some clinicians administer emulsion from a separate bottle in the infusion set, so that the TPN components are only mixed immediately prior to administration. Since the time taken for the mixture to flow from the mixing point to the catheter tip may be of the order of a minute, the mixture would still flocculate significantly even if this procedure was used. The only advantage of this method is that gross separation of emulsion and aqueous phases is not given the opportunity to occur. This is the most obvious practical disadvantage of flocculation; separation of the mixture into layers in the bag leads to nonuniform administration profiles. The bag can be remixed, but as discussed above, this will not deflocculate the mixture.

TPN Mixtures — The Future

The development of a theory of TPN stability is far from complete, even though it is likely that most of the separate interactions are now understood. The ultimate goal of research in the author's laboratory is to provide a means by which the stability and useful life of a mixture can be predicted without having to compound it and perform the appropriate stability measurements. This is being approached by two routes:

(1) A complete model of the interdroplet potentials in multicomponent media. This leads to the stability directly since it allows the energy well depth of the flocculated emulsion to be calculated. This is a computationally intensive procedure, but there are indications that it could provide a package that could be used transparently by the pharmacist on a day to day basis, in the same way that much simpler models are used at present.

(2) An empirical graphical approach based on a series of curves or nomograms. This is immediately suggested by Figs. 12 and 15; a knowledge of the monovalent/divalent electrolyte composition allows a flocculation rate to be measured from Fig. 12, which is modified by a value from Fig. 15. A further step would be needed to account for the effect of amino acids, and pH would have to be taken into account at the same stage as the electrolytes.

A considerable amount of further work is required before either of these methods will have any practical utility. However, it seems likely that it will be possible to accurately predict mixture stability, leading ultimately to a clinical benefit to the patient.

Acknowledgement

The author would like to thank Dr Jeremy Adler for obtaining the micrographs of flocculating emulsions.

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