International Journal of Pharmaceutics, 64 (1990) 217–222
Elsevier

IJP 02184

The electrokinetic properties of phospholipid stabilized fat emulsions. IV. The effect of glucose and of pH

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(Received 12 April 1990)
(Accepted 3 May 1990)

Key words: Emulsion; Stability; TPN; Parenteral nutrition; Interparticle potential

Summary

We have investigated the effects of glucose and pH on the flocculation of a parenteral fat emulsion (Intralipid 20%) by calcium and sodium ions. Glucose reduced the maximum flocculation rate caused by these ions by a considerable factor, which was not attributable solely to the increased viscosity of the medium. The point of zero charge was not changed by the addition of glucose. Alteration of the pH of the medium caused the emulsion point of zero charge to be shifted to lower calcium concentrations, while not affecting the maximum flocculation rate. The effect of glucose on the interdroplet potentials was calculated from the stability data. This allowed the possible changes in Van der Waals or hydration forces to be estimated, but it was not possible to distinguish which force component had been changed by the presence of glucose. The data suggest that glucose is highly important in determining the stability of fat emulsions in total parenteral nutrition mixtures.

Introduction

The destabilization of fat emulsions in TPN mixtures is a complex problem which is only beginning to be understood in detail. At Nottingham, theoretical and computational approaches to the prediction of TPN mixture stability are being developed (Washington, 1990a,b) which it is hoped will ultimately allow pharmacists to predict the stability of a mixture by calculation alone, without recourse to compounding. This requires a detailed understanding of the behaviour of the emulsion in a range of simple systems. Previous papers in this series have examined the flocculation of fat emulsions in simple electrolyte mixtures and have demonstrated that DLVO theory (Verwey and Overbeek, 1948) and its derivatives provide a good description of the results.

TPN mixtures contain a range of materials in addition to electrolytes, notably amino acids and glucose, which also modify the stability of the emulsion. Glucose alone has been shown to reduce the stability of fat emulsions, since it reduces the pH of the mixture (Black and Popovich, 1981). The surface potential of the emulsion depends on the pH, since H^+ is a potential-determining ion on phospholipid surfaces, with an isoelectric pH of 3.1 (Davis and Galloway, 1981). A reduction of pH would result in a decreased (less negative) surface potential and a more rapid rate of flocculation. In TPN mixtures the pH change is buffered

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to 6–7 by the amino acids present, and so this particular mode of destabilization would not be expected. Apart from any changes in pH which it may induce, glucose has previously been considered to be an inert material which does not affect TPN mixture stability. It will, of course, increase the viscosity of the mixture, which would be expected to reduce the flocculation rate as predicted by Smoluchowski (1917).

The present paper studies the effect of glucose on the flocculation of fat emulsions by monovalent and divalent cations. It is found that glucose produces a considerable stabilizing effect, which does not appear to be due to electrokinetic phenomena, and can only be understood in terms of the Van der Waals or solvation components of the interaction potentials between the droplets.

Materials and Methods

Intralipid 20% (batch no. 42320) was obtained from the Hospital Pharmacy (Queen's Medical Centre, Nottingham). NaCl and $CaCl_2$ were analar grade from May & Baker. Glucose was obtained as a 30% w/v solution from Laboratoires Aguettant (Lyons, France) and was adjusted to pH 7 with NaOH before use (this resulted in a final sodium concentration of less than 0.5 mM). The pH of the flocculating mixtures was controlled using 1 mM Hepes buffers adjusted to the desired pH using dilute HCl or NaOH. Flocculation rates were measured by turbidimetry as described previously (Washington and Davis, 1987). Zeta potentials were determined at 20 °C using a Malvern Zetasizer II.

All potential energy computations were performed using algorithms described previously (Washington, 1990b) using Microsoft Excel on an Apple Macintosh SE.

Results

The effect of increasing concentrations of glucose on the flocculation of Intralipid 20% by calcium is shown in Fig. 1. In the absence of glucose, the flocculation profile was in agreement with that observed previously (Washington, 1989, 1990a) with other batches of Intralipid 20%, and showed a critical flocculation concentration (CFC) between 1 and 2 mM calcium, and a point of zero charge (PZC) at a calcium concentration of 2 mM. Charge reversal prevented observable flocculation occurring above 6–8 mM $CaCl_2$. Increasing con-

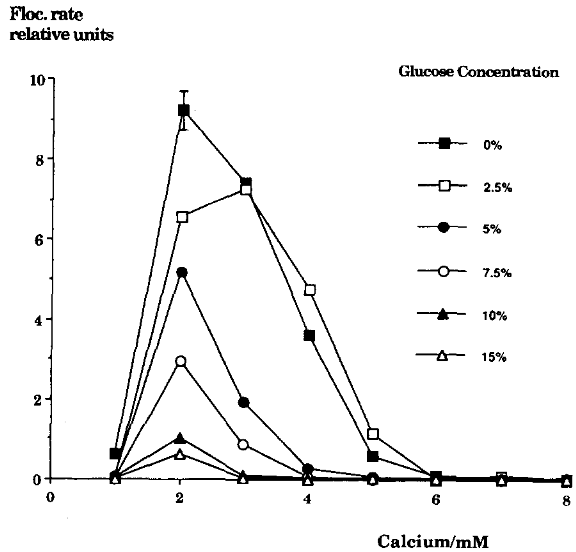

Fig. 1. Flocculation of Intralipid 20% by calcium (0–10 mM) in the presence of glucose (0–15% w/v).

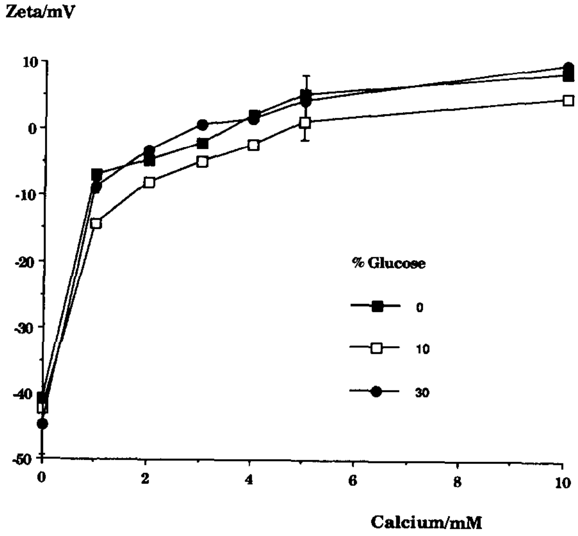

Fig. 2. Zeta potential of Intralipid 20% in the presence of $CaCl_2$ (0–10 mM) and glucose (0–30% w/v).

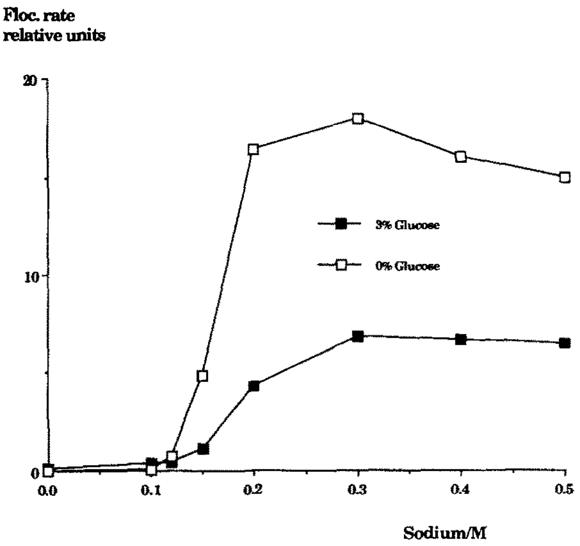

Fig. 3. Flocculation of Intralipid 20% by sodium (0–0.5 M) in the presence of glucose (0–3% w/v).

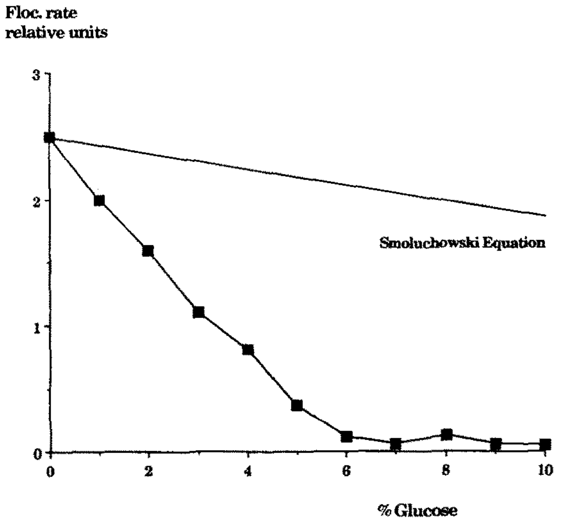

Fig. 4. Flocculation of Intralipid 20% by sodium (0.5 M) in the presence of glucose (0–10% w/v). Solid line is the prediction of the Smoluchowski equation.

centrations of glucose caused the peak flocculation rate to decrease in an approximately linear fashion, without changing the CFC or PZC, until flocculation was no longer detectable above a glucose concentration of 15% (all percentages quoted are w/v; 1% glucose is 55 mM).

The zeta potential of Intralipid 20% in increasing concentrations of glucose is shown in Fig. 2. Concentrations of glucose up to 30% did not cause any significant change in the zeta potential of the fat emulsion in $CaCl_2$ solutions of 0–10 mM concentration.

The effect of glucose on the flocculation of Intralipid 20% by sodium chloride (Fig. 3) shows similar trends to that observed with divalent electrolyte flocculation; 3% glucose decreased the flocculation rate at high sodium concentrations by approximately a factor of 2. The CFC was unchanged, and charge reversal was not observed, either in the presence or absence of glucose, due to the nonspecific nature of the adsorption of sodium to phospholipid surfaces. Fig. 4 shows the effect of glucose on the flocculation rate at a fixed sodium concentration of 0.5 M. The decrease in flocculation rate was approximately linear with increasing glucose concentration, until the flocculation became undetectable above a glucose concentration of 6%.

To contrast these effects with those caused by pH changes, Fig. 5 shows the effects of changing pH on the flocculation of Intralipid 20% by calcium. Increasing pH caused the CFC and PZC to move to higher calcium concentrations without

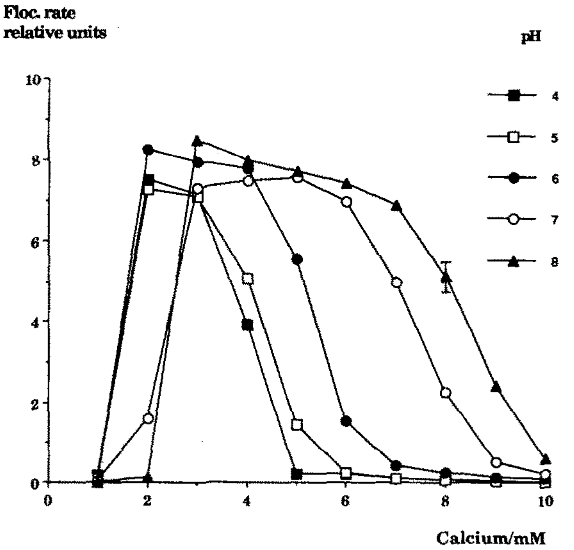

Fig. 5. Flocculation of Intralipid 20% by calcium (0–10 mM) as a function of pH (4–8).

any significant change in the peak flocculation rates at the PZC. The shift in the curves caused by the alteration in surface potential with pH is particularly noticeable in the charge-reversal region.

Discussion

Glucose significantly reduces the flocculation rate of fat emulsion in the presence of electrolytes. This effect is observed when the flocculation is induced by both monovalent and divalent cations, and is accomplished without a significant alteration in zeta potential as is demonstrated in Fig. 2. The fact that the PZC and CFC are unchanged in both electrolyte systems when glucose is added also confirms that this effect is independent of the electrostatic interaction between the emulsion droplets. Since 'normal regimen' TPN mixtures contain approx. 10% glucose, the stabilizing effect of this component in a clinical situation would be expected to be extremely important.

It is often suggested that glucose influences TPN stability via a change in pH, since autoclaved glucose solutions have a low pH. A reduction in pH would cause a change in surface ionisation of the phospholipid emulsifier; the pH-mobility profile of Intralipid has been measured by Davis and Galloway (1981) and demonstrates that a reduction in zeta potential would occur as the pH was reduced. This does not appear to be a significant factor in the present study. Firstly, the system was buffered to pH 7 to minimise such effects. Secondly, a decrease in pH caused by added glucose would reduce the zeta potential and thus increase the flocculation rate at a given electrolyte concentration; and finally such changes could not occur without a shift in the observed CFC and PZC. The effect of changing the pH on the flocculation rate is shown in Fig. 5; the changes in PZC and CFC are evident. Consequently it appears that, in the present experiments, glucose does not influence the stability of the system via the pH of the continuous phase. It should be noted that most TPN mixtures are also buffered to pH 6–7 by amino acids, and so it appears unlikely that glucose does in fact reduce TPN mixture stability. The absence of any change in the CFC or PZC also suggests that glucose does not alter stability by complexing the cations. This is chemically unlikely, and would in any event cause an alteration in the electrokinetic behaviour of the system.

The reduction in flocculation may be due to the increased viscosity of the medium, which reduces the droplet collision rate, in accord with the Smoluchowski equation:

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

where k_D is the diffusion-controlled flocculation rate, k is Boltzmann's constant, T is the absolute temperature, and η is the viscosity of the continuous phase. Viscosities of glucose solutions have been reported by Weast (1974). The upper line on Fig. 4 shows the flocculation rate which would be observed if glucose only influenced the rate through this viscous effect. A decrease in the flocculation rate is observed, but this accounts for only a small fraction of the total reduction in flocculation rate observed.

In order to explain the marked stabilization of the emulsion by glucose, we can use the flocculation data to investigate the possible changes in interdroplet potentials caused by glucose. This can be done using models described previously (Washington, 1990a). At present, we have only developed programs to perform this in solutions of nonspecifically adsorbing monovalent cations. Using the notation of our previous work, there are three variable parameters which could be influenced by glucose and thus alter the stability of the system; these are the continuous phase Hamaker constant A_{22} and the parameters of the hydration force, P_0 (the magnitude of the hydration force) and λ (its decay length in the solution). Changes in electrochemical parameters such as the surface charge density are unlikely, since they would alter the zeta potential. The change required in these parameters in order to produce the observed changes in flocculation rate was calculated. All other parameters are unchanged from those optimised previously for the Intralipid 20%/NaCl system. It is not possible to separate the effects of these parameters, and so the change required in each parameter was calculated on the

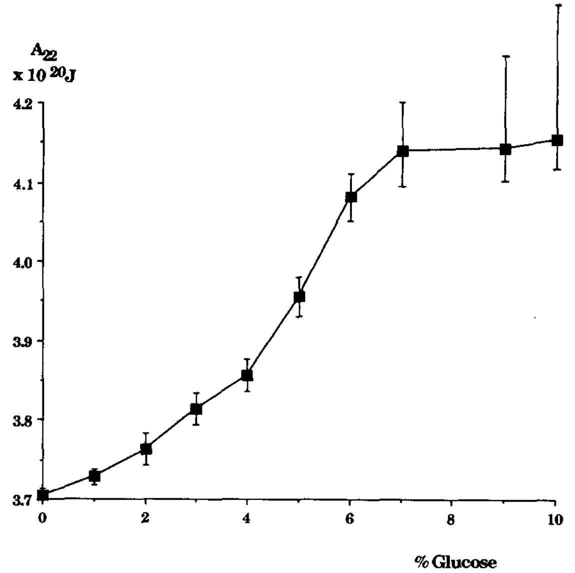

Fig. 6. Calculated change in the continuous-phase Hamaker constant A_{22} required to account for the observed stabilizing effect of glucose.

assumption that the other parameters are unchanged by the addition of glucose.

Fig. 6 shows a plot of the calculated change in the continuous phase Hamaker constant A_{22} with increasing glucose concentration. The value increases monotonically with increasing glucose concentration; the curve is distorted at higher glucose concentrations due to the difficulty of measuring extremely small flocculation rates close to the detection limit of the apparatus. It is believed that the curve would increase smoothly if it were possible to measure the flocculation rate with greater accuracy. A glucose concentration of 10% caused a calculated change in A_{22} of 13%; this does not seem unreasonable, but unfortunately the theory of Van der Waals forces in mixed solutions is not sufficiently well developed to confirm this figure.

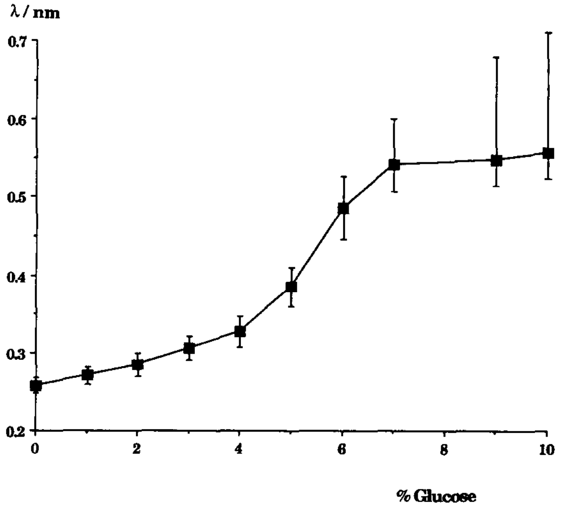

Fig. 7. Calculated change in the hydration force range parameter λ required to account for the observed stabilizing effect of glucose.

Fig. 7 shows similarly the corresponding change in λ with increasing glucose concentration. This also increases, from 0.26 nm in the absence of glucose, to 0.55 at 10% glucose. The physical interpretation of this is that the orientation of glucose at the droplet interface causes the solvent structure to be propagated further from the droplet surface.

The observed changes in flocculation rate could not reasonably be explained by variation of the parameter P_0, since this required to be increased by 5 or 6 orders of magnitude in order to account for the increased stability of the emulsion at a glucose concentration of 10%.

These figures represent the changes required in the individual parameters to account for the observed effect of glucose. In practice, it is possible that glucose alters both A_{22} and λ, and the changes in these parameters cannot be separated in the present experiment. It is also possible that amino acids exert their stabilizing effect in a similar manner, by influencing the surface steric forces or moderating the van der Waals force between the droplets. Experiments are in progress to test this hypothesis.

Conclusions

Glucose contributes a major and previously unsuspected stabilizing effect to fat emulsions in electrolyte solutions. Previously workers have attempted to rationalize the stability of TPN mixtures only on the basis of electrophoretic properties. Work from this laboratory has shown the importance of the remaining interdroplet force components on TPN stability; it now appears that at least one of the components of TPN mixtures influences the mixture stability via the steric or Van der Waals forces. The current work gives continued support to efforts to produce a deterministic model of emulsion stability in TPN mixtures.

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