International Journal of Pharmaceutics, 77 (1991) 57–63

ADONIS 037851739100365R

IJP 02564

The electrokinetic properties of phospholipid stabilized fat emulsions. V. The effect of amino acids on emulsion stability

C. Washington, M.A. Connolly, R. Manning and M.C.L. Skerratt

Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD (U.K.)

(Received 24 May 1991)
(Accepted 8 July 1991)

Key words: Emulsion; Stability; TPN; Parenteral nutrition; Amino acid

Summary

This study investigates the effect of amino acids, as commercial intravenous mixtures, on the flocculation of a parenteral fat emulsion (Intralipid 20%) by calcium ions. Flocculation was studied both by turbidimetry in dilute solutions, and by measurement of cream dimensions. The amino acid solutions stabilised the emulsion/electrolyte mixtures to a considerable degree; two separate influences were apparent in the flocculation profile. The peak flocculation rate (or maximum cream depth) of the mixtures was depressed by the addition of amino acids, suggesting that the interdroplet Hamaker constant was changed in the systems. In addition, amino acids ionized into the predominantly negative form acted as complexing agents for calcium, shifting the flocculation curve to higher calcium concentrations. The effect of calcium binding was only marked at high pH (8–9) and suggests that amino acids, like glucose, stabilize TPN mixtures primarily by decreasing the attractive component of the interdroplet force.

Introduction

Parenteral fat emulsions such as Intralipid are often administered by mixing with a range of other nutritional solutions in a single container prior to administration; these mixtures are usually termed all-in-one (AIO) or total parenteral nutrition (TPN) mixtures. The interaction of the emulsion with the electrolyte component of the mixture leads to instability, manifested as droplet flocculation and ultimately coalescence to produce free oil, a process which is commonly supposed to be deleterious to patient health. Consequently, an understanding of the basis of instability in TPN mixtures is of considerable value in their formulation and compounding, since it will allow unstable mixtures to be identified, and shelf life to be predicted.

Previous papers from this group have devoted some effort to developing theoretical and computational approaches to the prediction of TPN mixture stability (Washington, 1990a,b). The underlying approach is to investigate the behaviour of simpler model systems containing fewer com-

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

ponents, which can then be used to predict the behaviour of more complex mixtures. Consequently, previous studies have examined the flocculation of fat emulsions in electrolytes and simple electrolyte mixtures (Washington, 1990a), the effect of pH and of glucose (Washington et al., 1990) and have demonstrated that DLVO theory (Verwey and Overbeek, 1948) and its derivatives provide a good description of the results, with flocculation being largely controlled by the electrostatic potential on the droplet surface.

The present paper investigates the effect of amino acids on the flocculation of fat emulsions by electrolytes. These are the only major components of TPN mixtures which have not been studied to date by this group. Amino acids are known to be important in the stabilization of TPN mixtures (e.g. Barat et al., 1987), although systematic data are scarce. We have studied the effects of commercial parenteral amino acid solutions, which contain a wide range of different amino acids, on flocculation rate and cream depth in mixtures containing Intralipid 20%, amino acid solution, and a single monovalent and divalent electrolyte (sodium or calcium). It was also necessary to study the effect of pH on the mixture stability, in order to establish the importance of amino acid ionization. Experiments studied both the commercial 'Vamin 14 EF' and 'Synthamin 14 EF' amino acid mixtures; it was of course necessary to use the electrolyte-free formulations in these studies.

Previous studies have been based largely on measurement of flocculation rate in diluted emulsions by turbidimetric means, a technique which is rapid and convenient. However, it is commonly observed that TPN mixtures do not flocculate to completion, but form an equilibrium state in which a fraction of the emulsion forms a cream layer, and the remainder is unflocculated and remains well dispersed. There is no assurance that the initial rate of flocculation, as measured by turbidimetry, is correlated with the position of this equilibrium in the more concentrated mixtures. Consequently, the present experiments study both the initial flocculation rate and the dimensions of the cream layer at equilibrium, in order to clarify this point.

Materials and Methods

Intralipid 20% (Kabi Pharmacia, batch no. 42320), Vamin 14 EF (Kabi Pharmacia, batch no. 90061) and Synthamin 14 EF (Baxter Health Care, batch no. 840614A01) were obtained from the Hospital Pharmacy, Queen's Medical Centre, Nottingham. Sodium and calcium chlorides were analar grade from May and Baker.

The pH of the flocculating mixtures was controlled by the addition of small amounts of hydrochloric acid or sodium hydroxide of a sufficiently high concentration (normally 2 M) to avoid altering the mixture volume or ionic strength significantly. This approach is useful since it exploits the buffering capacity of the amino acids for pH control without the addition of other buffer materials.

Flocculation rates were measured by turbidimetry using apparatus described previously (Washington and Davis, 1987). In order to measure flocculation rates in media containing both calcium and amino acids, the calcium chloride and amino acid solutions were mixed in the desired proportions, and any necessary volume adjustments made with distilled water, to provide solutions containing 0–20 mM calcium chloride and 0–40% v/v amino acid solution. This stock solution was then mixed with an equal volume of emulsion (diluted to an oil phase volume of 0.1%) immediately prior to turbidimetric measurement. The final calcium and amino acid concentrations were thus half of those in the stock solutions. The turbidity change of the mixtures was measured for approx. 1–2 min and the flocculation rate expressed as the slope of turbidity vs time.

Creaming behaviour was measured in mixtures containing undiluted Intralipid 20% (10 ml), calcium chloride and amino acid solutions, and water to 20 ml. The samples contained the same amino acid and calcium concentrations as those used in the turbidimetric studies. These were placed in glass vials and left undisturbed for 24 h at room temperature (20 ± 5°C) prior to measurement of cream depth, recorded as a percentage of total mixture depth. No significant alteration in cream depth was observed in any of the mixtures after 24 h.

Results

Figs 1–6 show the effect of increasing concentrations of Vamin 14 EF on the flocculation of Intralipid 20% by calcium ions. Figs 1 (pH 4), 2 (pH 6) and 3 (pH 8) show the stability as measured by cream depth, while Figs 4 (pH 4.5), 5 (pH 6) and 6 (pH 9) illustrate the stability of the same system measured in diluted emulsion systems using turbidimetry. At pH 4 and 4.5, the emulsion flocculation rates are reduced without significant change in the critical flocculation concentration (CFC) or the point of zero charge (PZC) as the amino acid concentration is increased. At pH 6 the flocculation rates are reduced, and the profiles are shifted slightly to higher calcium concen-

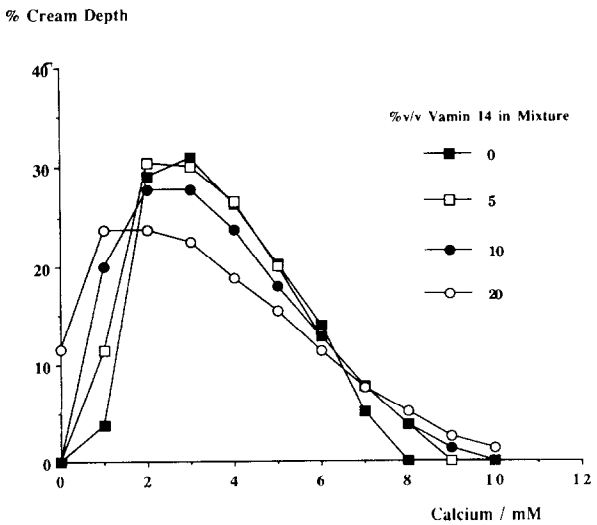

Fig. 1. Cream depth of Intralipid 20% in mixtures containing calcium (0–10 mM) and Vamin 14 EF (0–20% v/v) at pH 4.

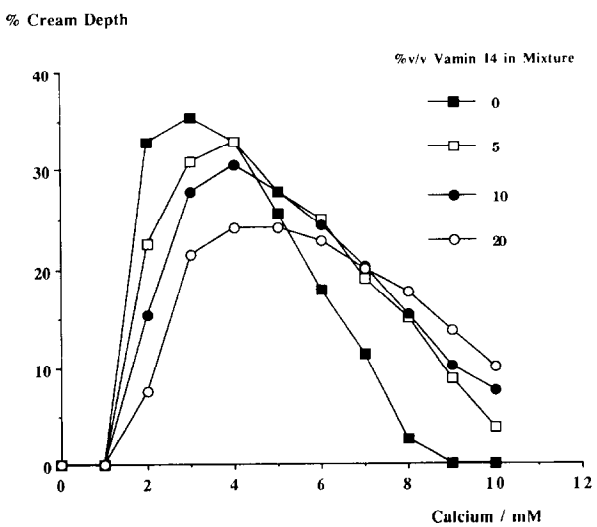

Fig. 2. Cream depth of Intralipid 20% in mixtures containing calcium (0–10 mM) and Vamin 14 EF (0–20% v/v) at pH 6.

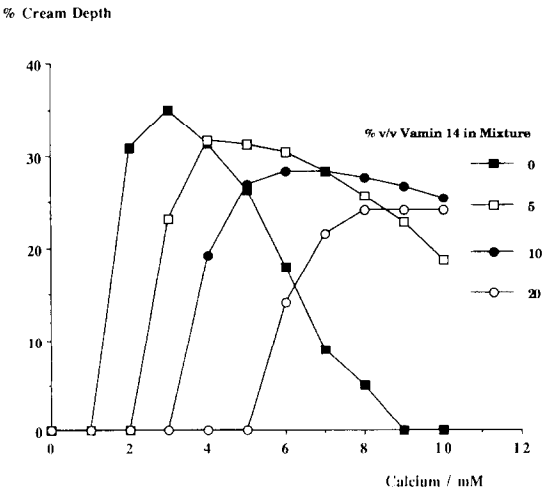

Fig. 3. Cream depth of Intralipid 20% in mixtures containing calcium (0–10 mM) and Vamin 14 EF (0–20% v/v) at pH 8.

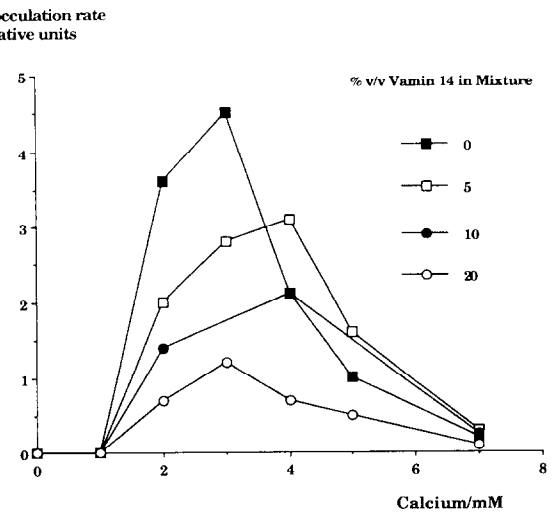

Fig. 4. Flocculation rate (turbidimetric) of Intralipid 20% by calcium (0–10 mM) in the presence of Vamin 14 EF (0–20% v/v) at pH 4.5.

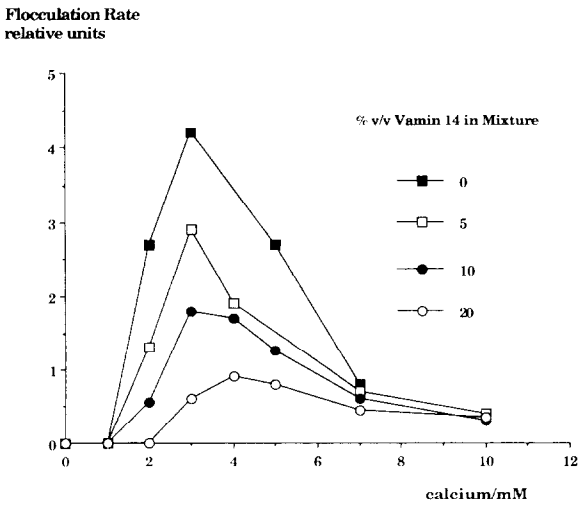

Fig. 5. Flocculation rate (turbidimetric) of Intralipid 20% by calcium (0–10 mM) in the presence of Vamin 14 EF (0–20% v/v) at pH 6.

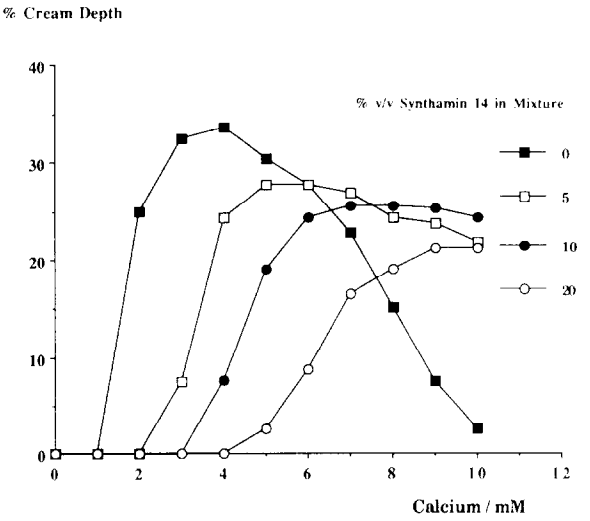

Fig. 7. Cream depth of Intralipid 20% in mixtures containing calcium (0–10 mM) and Synthamin 14 EF (0–20% v/v) at pH 8.

trations. This shift is most evident at pH 8 and 9, in which only the initial stages of flocculation can be detected at the highest amino acid concentrations, since the main part of the flocculation curve has been shifted out of the calcium concentration range studied.

Fig. 7 shows a similar measurement using the amino acid solution Synthamin 14 EF instead of Vamin 14 EF at pH 8. The profile is similar to the corresponding result for Vamin 14 EF (Fig. 3).

Fig. 8 shows the effect of increasing concentrations of the amino acid solution Vamin 14 EF on

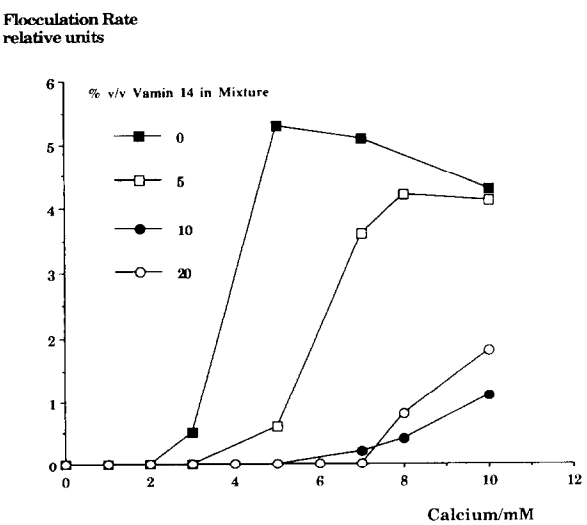

Fig. 6. Flocculation rate (turbidimetric) of Intralipid 20% by calcium (0–10 mM) in the presence of Vamin 14 EF (0–20% v/v) at pH 9.

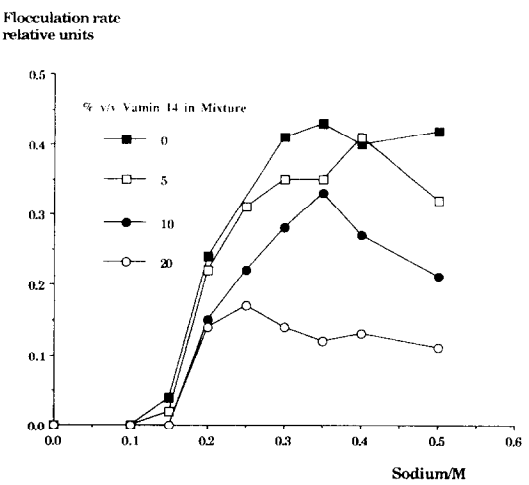

Fig. 8. Flocculation rate (turbidimetric) of Intralipid 20% by sodium (0–0.5 M) in the presence of Vamin 14 EF (0–20% v/v) at pH 6.

the flocculation induced by sodium at pH 6. There is no significant change in the CFC of 0.15 M, and the presence of the amino acid solution monotonically depresses the flocculation rate at any particular concentration of sodium ions.

Discussion

The correlation between the stability of all the mixtures in both concentrated (cream depth measurement) and dilute (turbidimetric) emulsion studies is extremely good. This increases confidence in the relevance of previous measurements in dilute emulsion systems to the behaviour of TPN mixtures in which a much larger proportion of emulsion is present, and demonstrates that the equilibrium cream formation is well correlated with the kinetic flocculation behaviour of the mixtures. This is reassuring, particularly since the mechanism by which partial cream formation occurs is presently unclear. One possibility (Washington, 1990b) is that the shallow nature of the interdroplet potential well allows an equilibrium between bound and unbound droplets; however, phase separation would disturb this equilibrium. An additional possibility is that the ion activities are different in the cream and the unflocculated mixture. Further experiments are required to clarify this matter.

The flocculation of the emulsions in the absence of amino acids is in good agreement with previous turbidimetric measurements (Washington, 1990a; Washington et al., 1990). These indicate that, at pH 6–7, the CFC of calcium for Intralipid 20% is approx. 2 mM, and that the PZC occurs at 3–4 mM calcium, with charge reversal evident at higher calcium concentrations. This profile is shifted slightly to lower calcium concentrations at lower pH, and significantly to higher calcium concentrations at higher pH.

Two separate effects are visible as the amino acid concentration in the systems is increased. Firstly, the peak flocculation rates are decreased in an approximately monotonic fashion with increasing amino acid concentration, an effect that is most evident at the PZC. This is a similar effect to that observed for increasing concentrations of glucose (Washington et al., 1990) and which appears to be due to a decrease in the interdroplet Hamaker constant due to the alteration of the dispersive properties of the interdroplet medium. This effect cannot be due to changes in the electrostatic part of the interdroplet potential, since this would alter the position of the CFC or PZC in these systems. This aspect of stabilization by the amino acids does not appear to be strongly dependent on pH, since the peak flocculation rate at the PZC is depressed to a similar extent by a given amino acid concentration over the entire pH range 4–8. This stabilizing effect is also observed when sodium is used as the flocculating cation. Sodium is adsorbed nonspecifically to the droplet surface, and is not strongly bound by amino acids, thus supporting the hypothesis that amino acids are stabilizing via their effect on the Van der Waals' forces and not the electrostatic potential.

Secondly, the flocculation profiles are significantly shifted to higher calcium concentrations at pH 8 and above. This trend is only marginally visible at pH 6. It is likely that this is due to the complexing of calcium by the negatively charged amino acids, thus reducing the effective calcium activity available for surface adsorption. Calcium adsorption by amino acids is known to be important in TPN mixtures, since it can, for example, alter the critical precipitation concentration for calcium phosphate (Allwood, 1987). The magnitude of the reduction in flocculation rate is similar to that observed for glucose in previous studies, and since these solutions are major components of TPN mixtures, we would expect this stabilizing effect to be highly significant in most all-in-one regimens.

Similar trends are visible when the amino acid mixture Synthamin 14 EF is used in place of Vamin 14 EF. The amino acid compositions of these mixtures are given in Table 1. Both mixtures consist of predominantly neutral amino acids, with isoelectric points in the range 5.0–6.5, with a smaller proportion of basic or acidic amino acids; the only major difference is that Synthamin contains no acidic amino acids.

The large number of components in the amino acid mixtures currently prevents the discussion of

TABLE 1

Compositions of amino acid solutions (g / 1000 ml)

Amino acid	Vamin 14 EF	Synthamin 14 EF
Alanine	12.0	17.6
Arginine	8.4	9.78
Aspartic acid	2.5	0
Cysteine	0.42	0
Glutamic acid	4.2	0
Glycine	5.9	8.76
Histidine	5.1	4.08
Isoleucine	4.2	5.1
Leucine	5.9	6.2
Lysine	6.8	4.93
Methionine	4.2	3.4
Phenylalanine	5.9	4.76
Proline	5.1	5.78
Serine	3.4	4.25
Threonine	4.2	3.57
Tryptophan	1.4	1.53
Tyrosine	0.17	0.34
Valine	5.5	4.93
% Neutral	74.3	82.6
% Acidic	7.9	0
% Basic	17.8	17.4

these results on the basis of the properties of individual amino acids. Fortunately, the stabilizing effects of the mixtures appear to be explicable by reference to the predominant neutral amino acids alone. At pH 4 and 6, the majority of the amino acids are in their neutral (zwitterionic) state, and only weak calcium binding occurs, probably due to those amino acids with the lower isoelectric points. At pH 4, the most acidic amino acids would be positively charged, and these may complicate the simple model by adsorbing to the emulsion droplet and reducing its zeta potential. This behaviour is suggested in Fig. 1, in which 20% Vamin 14 EF at pH 4 is slightly destabilizing in the absence of calcium. At pH 8 and above, many of the amino acids are predominantly negatively charged, and thus the calcium binding effect is marked.

Since calcium binding does not appear to be important at pH 6 but is significant at pH 8, it may be suggested that TPN mixtures with a high pH (such as those made using the Aminoplex (Geistlich) solutions, with a pH of 6.8–7.0) would display superior stability to more acidic regimens. This would only be true as long as the total electrolyte load was sufficiently low that the emulsion was not in the charge-reversed region. Raising the pH from, e.g., 5.5 to 7.0 also increases the zeta potential of the unmixed emulsion from approx. −35 to −42 mV (figures vary depending on emulsion batch and source), which would have an additional stabilizing effect.

We have already demonstrated that pH is of central importance in determining TPN mixture stability, since it controls the ionization of the surface phospholipids (Davis and Galloway, 1981) and the subsequent surface adsorption of ions (Washington, 1990b). It now appears that pH has the additional role of modulating the stabilization of the emulsion caused by amino acids, particularly in mixtures which have been adjusted to a pH near neutral. The effect of increased pH on TPN mixture stability depends on whether or not the emulsion droplets in the mixture are charge reversed. A mixture in which the emulsion is negatively charged would be stabilized by an increase of pH, since it would increase surface ionization and hence surface charge, and increase the potential calcium binding capacity of the amino acids, thus decreasing calcium activity in solution. Alternatively, a mixture in which the emulsion droplets were charge reversed would be destabilized by an increase in pH, since the amino acid binding would compete for calcium bound to the droplet surface. At present, the technical difficulty of measuring the small zeta potential of fat emulsions in TPN mixtures (as opposed to dilute model systems) does not allow the mobility of droplets in a particular mixture to be accurately determined, although preliminary studies suggest that, in many mixtures, the emulsion droplets are near the PZC.

Conclusions

Amino acid solutions are shown to stabilize TPN mixtures by a combination of calcium complexation and reduction in attractive Van der Waals' forces; the latter is the major mechanism contributing to the stabilizing effects observed in

clinical regimens. The present paper completes our initial studies of the interactions between the components of TPN mixtures, and the framework of fat emulsion stability in these systems is now broadly understood. We hope that, in the near future, it will be possible to produce a simple algorithm for predicting mixture stability, based on this and our previous studies.

References

Allwood, M.C., The compatibility of calcium phosphate in paediatric TPN infusions. *J. Clin. Pharm. Ther.*, 12 (1987) 293–301.

Barat, A.C., Harrie, K., Jacob, M., Diamantidis, T.G and McIntosh, N.L., Effect of amino acid solutions on total nutrient admixture stability. *J. Parenter. Enteral Nutr.*, 11 (1987) 384–388.

Davis, S.S. and Galloway, M., Effects of blood plasma components on the properties of an intravenous fat emulsion. *J. Pharm. Pharmacol.*, 33 (1981) (Suppl.) 88P.

Verwey, E.J.W. and Overbeek, J.T.G., *Theory of Stability of Lyophobic Colloids*, Elsevier, Amsterdam, 1948.

Washington, C., The electrokinetic properties of phospholipid-stabilized fat emulsions. II. Droplet mobility in mixed electrolytes. *Int. J. Pharm.*, 58 (1990a) 13–18.

Washington, C., The electrokinetic properties of phospholipid-stabilized fat emulsions. III. Interdroplet potentials and stability ratios in monovalent electrolytes. *Int. J. Pharm.*, 64 (1990b) 67–73.

Washington, C. and Davis, S.S., Aging effects in parenteral fat emulsions: the role of fatty acids. *Int. J. Pharm.*, 39 (1987) 33–37.

Washington, C., Athersuch, A. and Kynoch, D.J., The electrokinetic properties of phospholipid-stabilized fat emulsions. IV. The effect of glucose and of pH. *Int. J. Pharm.*, 64 (1990) 217–222.