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Compatibility of Intravenous Fat Emulsions with Prodrug Amino Acids

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Abstract—Three prodrugs, *N*-α-acetyl-L-arginine, *N*-α-acetyl-L-histidine and *N*-α-acetyl-L-lysine have been examined to see if they could induce significant changes in the stability of an intravenous fat emulsion, the stability being evaluated in terms of physicochemical measurements such as particle size distribution, surface tension, pH and zeta potential. The acetyl amino acids had an excellent stabilizing effect on the emulsion compared with amino acids without acetyl groups.

Investigations into the compatibility of intravenous fat emulsions with carbohydrates, electrolytes and amino acids have been made (Whateley et al 1984; Sayeed et al 1986). In our previous study (Takamura et al 1984), the isoelectric point (pI) of amino acids used as intravenous additives, and the pH of the systems, were found to be factors controlling the stability of fat emulsions which are sensitive to basic amino acids in a neutral solution. When a basic amino acid with a positively charged group is adsorbed onto a negatively charged oil droplet surface, the electrostatic repulsion between two droplets weakens in the fat emulsion.

We have examined three acetyl amino acids, namely *N*-α-acetyl-L-arginine, *N*-α-acetyl-L-histidine and *N*-α-acetyl-L-lysine which may be considered for use as prodrugs for intravenous additive purposes, to replace the parent amino acids, arginine (pI = 10·76), histidine (pI = 7·64) and lysine (pI = 9·47) which have high isoelectric points. The compatibility of fat emulsions with these amino acid prodrugs has been investigated along with various physicochemical properties in relation to particle size distribution (Ludvik & Durdil 1983), surface tension (Toida et al 1978), pH (Dawes & Groves 1978) and zeta potential (Kawilarang et al 1980).

Materials and Methods

Amino acids and their prodrugs

L-Arginine monohydrochloride (Arg), L-histidine monohydrochloride monohydrate (His) and L-lysine monohydrochloride (Lys), selected as the amino acids for intravenous additives, were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). *N*-α-Acetyl-L-arginine monohydrochloride (Ac-Arg), *N*-α-acetyl-L-histidine monohydrochloride monohydrate (Ac-His) and *N*-α-acetyl-L-lysine monohydrochloride (Ac-Lis) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used as the prodrug of each amino acid. A 10 (w/v)% amino acid solution (ES-Polytamin, Daigo Nutritive Chemicals, Ltd.), containing 11 amino acids (see Fig. 5), was also used as a fat emulsion additive.

Admixture of amino acids with fat emulsion

Details of the preparation of the fat emulsion have been given (Takamura et al 1984). Briefly, the emulsifier (egg phospholipid) was dissolved in the oil phase, maintained at 80°C in a tank, and water which had been preheated to 80°C was added to the solution. The agitator used was an autohomomixer (Type IM, Tokushukika Co., Ltd., Osaka, Japan): the impeller speed was maintained at 11 200 rev min^{-1}. The resulting coarse emulsion was introduced rapidly into the homogenizer (Model 15M-8TA, Gaulin Co., Ltd, Mass, USA) at 4500 psi.

Each amino acid was used as a 1% (w/v) solution. The fat emulsion was mixed with an equal volume of either the amino acid solution or with a commercial 10 (w/v)% amino acid solution containing 11 different kinds of amino acids. Fat emulsion, admixed with an equal volume of distilled water, containing 2·5 (w/v)% glycerol, was used as a control. The mixture of fat emulsion with each amino acid solution was stored at 20 ± 0·1°C for 14 days.

Measurement of emulsion stability

The emulsified stability was evaluated by measurements of particle size distribution, surface tension, pH and zeta potential, parameters previously shown to be useful in stability studies (Noro et al 1979).

The particle sizes and their distribution in the fat emulsion were observed with a specific fixation technique using a scanning electron microscope (Model JSM T200, JEOL Ltd., Tokyo, Japan) and determined with a Coulter Nanosizer (Type N4, Coulter Electronics Inc., Hialeah, Fla, USA) by laser light scattering, respectively. The surface tension of the fat emulsion was measured with a Du-Nouy tensiometer. The hydrogen ion concentration of the sample emulsion was determined using a pH meter. The zeta potential (Laser Zee Model 500, Penkem Inc., New York) was calculated from the mean electrophoretic mobility of the oil droplets. Measurements of zeta potential were performed in the same continuous phase.

Results and Discussion

Evaluation of emulsion stability by particle size distribution

Fig. 1 shows scanning electron micrographs of fat emulsions taken either immediately or 14 days after addition of Arg or Ac-Arg to a level of 0·5% (w/v). The particle size did not change significantly, but when Arg was admixed, some large

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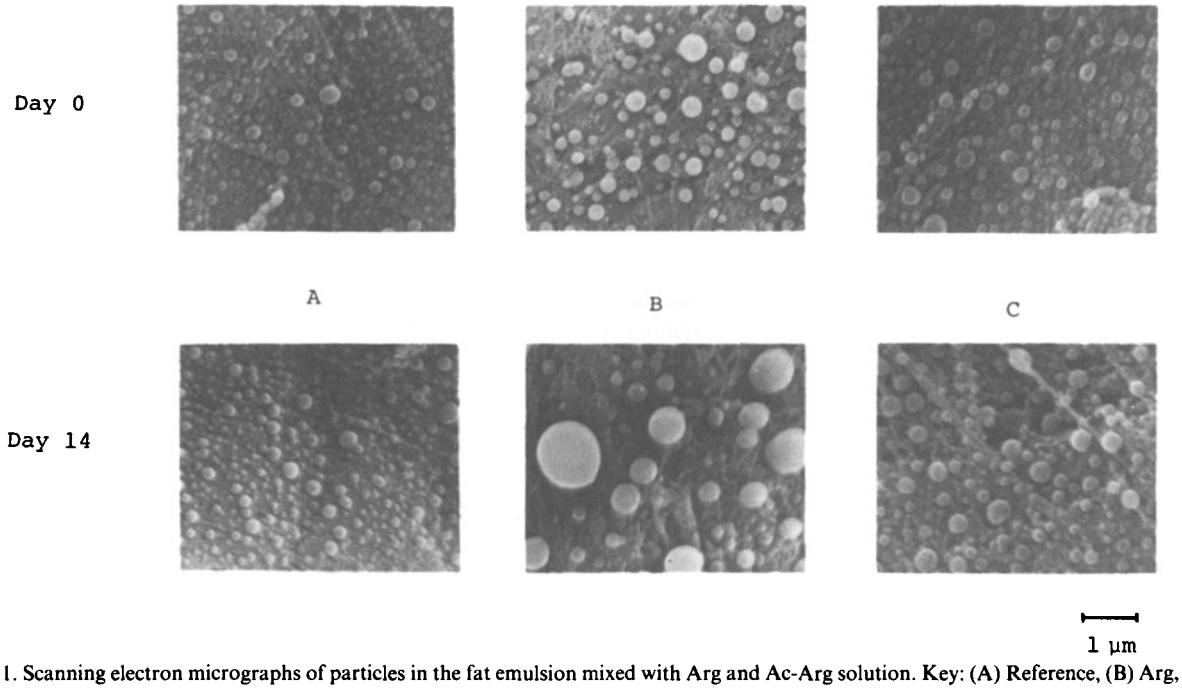


FIG. 1. Scanning electron micrographs of particles in the fat emulsion mixed with Arg and Ac-Arg solution. Key: (A) Reference, (B) Arg, (C) Ac-Arg.

particles were observed at 14 days, but there was no change with Ac-Arg.

The typical electron microscopic size distribution for the fat emulsion determined for 1000 emulsion droplets on micrographs shown in Fig. 1 is shown in Fig. 2 and the mean diameters listed in Table 1. For the Arg and Ac-Arg samples, the distribution curves and the mean diameters were similar initially and there was no significant difference in the particle size distribution curves at 0 and 14 days after addition of Ac-Arg (Fig. 2B), there was a difference when Arg was used (Fig. 2A).

The mean diameters of the droplets were not significantly changed after admixing any of the three acetyl amino acid solutions with the fat emulsion, but they increased markedly after 14 days when the corresponding amino acid solution was mixed into the fat emulsion.

Evaluation of emulsion stability by physicochemical measurements

With the three amino acids, the surface tension of the fat emulsion decreased markedly at the early stage of storage, and then levelled off after 10 days (Fig. 3). This could be because some large oil droplets appeared and flocculated to the surface of the emulsion. On the other hand, the tension remained constant throughout the 14 days in the emulsion mixed with the acetyl amino acids.

Fig. 4 shows the effect of amino acids and acetyl amino acids on the pH of the fat emulsion. The Lys and Arg caused

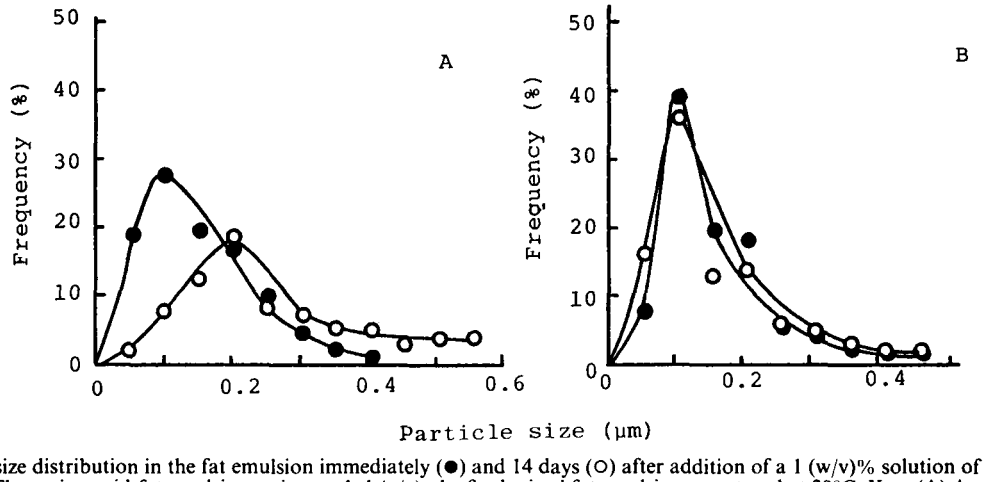


FIG. 2. Particle size distribution in the fat emulsion immediately (●) and 14 days (○) after addition of a 1 (w/v)% solution of amino acid in distilled water. The amino acid-fat emulsion ratio was 1:1 (v/v); the final mixed fat emulsion was stored at 20°C. Key: (A) Arg, (B) Ac-Arg.

Table 1. Mean diameter of droplets in the fat emulsion mixed with various amino acids. The amino acid solution-fat emulsion ratio was 1 : 1 (v/v); the final mixed fat emulsion was stored at 20°C for 14 days.

	Mean diam, nm			Mean diam, nm	
Solution	Day 0	Day 14	Solution	Day 0	Day 14
Arg HCl	210±57	341±100	Ac-Arg	217±50	226±49
His HCl	214±67	527±170	Ac-His	215±61	221±62
Lys HCl	214±51	325±98	Ac-Lys	213±58	229±47
Syn. A.A.	214±60	404±94	Ac-Syn. A.A.	215±53	241±41
Reference	211±50	210±52			

Abbreviations; Arg HCl: L-arginine HCl, Ac-Arg: acetyl arginine, His HCl: L-histidine HCl, Ac-His: acetyl histidine, Lys HCl: L-lysine HCl, Ac-Lys: acetyl lysine, Syn. A.A.: synthetic amino acid, Ac-Syn. A.A.: acetyl synthetic amino acid

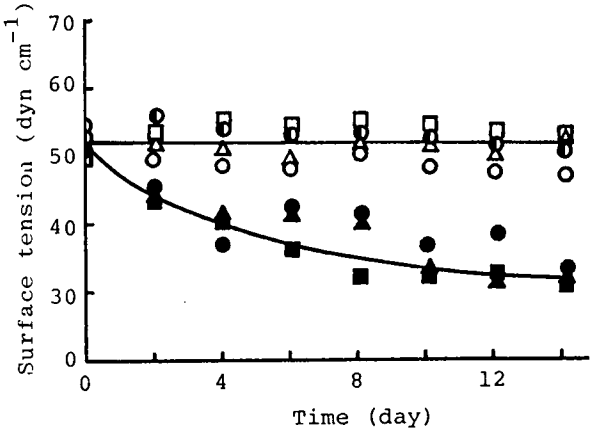


FIG. 3. Effect of addition of various 1 (w/v)% amino acid solutions on the surface tension of the fat emulsion. Key: (◐) Reference, (▲) Arg, (■) His, (●) Lys, (△) Ac-Arg, (□) Ac-His, (○) Ac-Lys.

a decrease in the pH of each amino acid/fat emulsion. However, the three acetyl amino acids did not significantly alter the pH. As each amino acid solution was prepared with double distilled, decarbonated water (pH 7) rather than with buffer, it altered the pH of the mixing system. Consequently, the mixtures started at different pH values.

The zeta potential ranged between −40 and −50 mV, and showed no significant change during the 14 day period after addition of amino acids or acetyl amino acids.

Effect of total amino acids on emulsion stability

Fig. 5 shows that particle size distribution curves of the two total solutions of amino acids and acetyl amino acids measured by the Coulter Nanosizer.

No significant differences in the size distribution curves were found between those obtained immediately and those at 14 days after total acetyl amino acids were admixed with the fat emulsion. However, considerable differences were observed in the size distribution curves between those obtained immediately and those at 14 days after amino acids were admixed with the fat emulsion.

The changes in the surface tension of the fat emulsion after admixing with 10 (w/v)% total solutions of amino acids and acetyl amino acids are shown in Fig. 6. For the amino acids, the surface tension values decreased until 6 days, and then levelled off, while for the total acetyl amino acid solutions, the values did not appreciably alter after 14 days of storage.

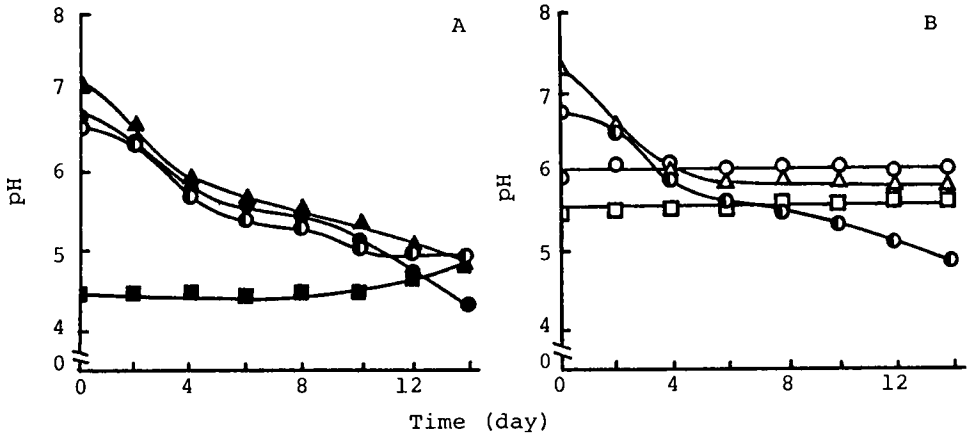


FIG. 4. Effect of addition of 1 (w/v)% solutions of amino acid (A), and acetyl amino acid (B) on the pH of the fat emulsion. Key: (◐) Reference, (▲) Arg, (■) His, (●) Lys, (△) Ac-Arg, (□) Ac-His, (○) Ac-Lys.

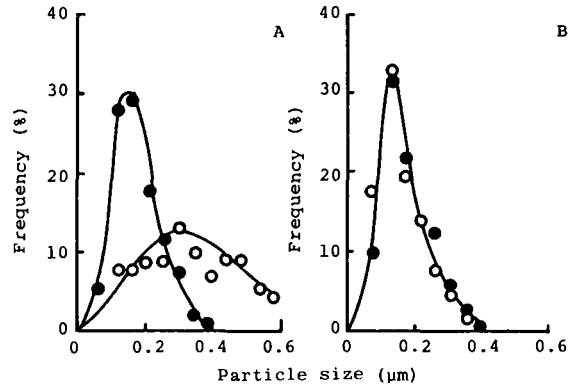


FIG. 5. Particle size distribution in the fat emulsion immediately (●) and 14 days (○) after adding a 10 (w/v)% solution of synthetic amino acid in distilled water. The synthetic amino acid-fat emulsion ratio was 1:1 (v/v); the prepared final act emulsion was stored at 20°C. Key: (A) The mixture of 11 amino acids L-arginine HCl* 2·00, glycine 2·98, L-histidine HCl·H_2O* 1·00, L-isoleucine 1·92, L-leucine 2·18, L-lysine HCl* 2·88, L-methionine 1·92, L-phenylalanine 1·28, L-threonine 1·28, L-tryptophan 0·64, L-valine 1·92 g. Total 20·00 g in 200 mL. (B) The mixture of three acetyl amino acids (Ac-Arg, Ac-His, Ac-Lys) and 8 amino acids without the asterisk above.

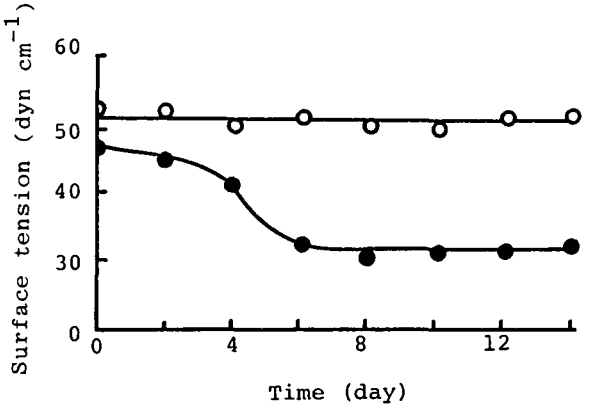
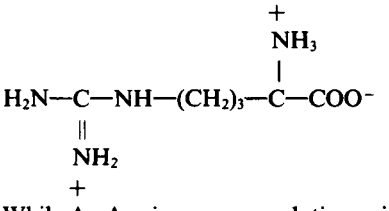


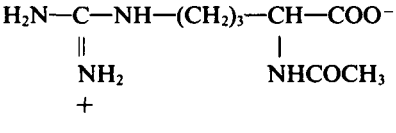
FIG. 6. Effect of addition of 10 (w/v)% synthetic amino acid solution on the surface tension of the fat emulsion. The amino acid solution (10%, w/v)-fat emulsion ratio was 1:1 (v/v); the prepared final fat emulsion was stored at 20°C. Key: (●) The mixture of 11 amino acids shown in Fig. 5. (○) The mixture of three acetyl amino acids (Ac-Arg, Ac-His, Ac-Lys) and 8 amino acids as in Fig. 5.

Dissociation of amino acids and acetyl amino acids

Arginine in aqueous solution exists generally as a positively charged ion:



While Ac-Arg in aqueous solution exists as



Thus, it is considered that the positively charged NH_3^+ group is able to circumvent any interference due to the presence of the negatively charged COO^- group at an intermolecular site. Due to similar ionic circumstances, His and Lys also exist as positively charged ions (Takamura et al 1984). On the other hand, Ac-His and Ac-Lys exist as neutral molecules in aqueous solution.

Colloidal particles are known to interact with each other through van der Waals' forces. On the other hand, the charge on emulsion droplets arising from ionization of the emulsifying agent (lecithin) on their surfaces will result in repulsion when these interacting particles have the same surface charge and potential. The DLVO theory of stability of lyophobic colloids developed by Derjaguin and Landau and by Verwey and Overbeek combines the latter electrostatic repulsive energy and the former van der Waals' attractive energy. The stability of the emulsion can be evaluated in terms of the total potential energy of these interactions. The addition of amino acid to the emulsion will give rise to a reduction in repulsive energy because the charge of the amino acid in the system differs from that on the surface of the emulsion droplets. Davis & Galloway (1981) reported that at pH values above 5·5, the fat emulsion carries a negative charge. This negative charge arises from ionization of the various minor components (e.g. phosphatidyl ethanolamine, phosphatidyl inositol, etc.) (Bangham 1968) present in egg lecithin as an emulsifier. Since Arg exists as a positively charged ion in aqueous solution, the charge of the oil particles will be neutralized when an Arg molecule is added. As a result, coalescence or creaming of the oil particles frequently occurs in the fat emulsion, because the electrostatic repulsion between the oil droplets weakens.

The electric effect of Ac-Arg on the negatively charged oil droplets surface is considered to be weak, because Ac-Arg exists as neutral molecule in aqueous solution. Thus, the fat emulsion remains stable when acetyl amino acid is admixed with it.

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