Study of Intravenous Hyperalimentation: Effect of Selected Amino Acids on the Stability of *Intravenous* Fat Emulsions

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Abstract □ In an attempt to resolve the conflicts relevant to intravenous fat emulsion compatibility two amino acids, L-valine and L-histidine monohydrochloride monohydrate, were chosen as intravenous additives, and these substances were investigated as to whether they could induce significant changes in the physicochemical properties of an intravenous soybean oil emulsion. The stability of the fat emulsion was evaluated in terms of gross visual observation, particle size distribution, surface tension, pH, and the zeta potential. It was found that (*a*) no significant difference was produced on the particle size distribution up to 7 d after admixing, (*b*) the mean diameter of oil globules was ~0.21–0.24 μm, and (*c*) the fat emulsion was sufficiently stable, with a zeta potential ranging between −30 and −60 mV. The relationship of the isoelectric point (pI) of the amino acids and the pH of the buffer solutions was determined to be one of the most important factors in the stability of the fat emulsion.

Keyphrases □ Fat emulsions—stability, effect of L-valine and L-histidine monohydrochloride monohydrate additions, buffers □ Stability—long-term, fat emulsions admixed with L-valine and L-histidine monohydrochloride monohydrate and buffers □ Amino acids—L-valine, L-histidine monohydrochloride monohydrate, effect on fat emulsions, long-term stability of admixtures

Intravenous hyperalimentation has been developed for patients who cannot take food orally following surgery (1). Total parenteral nutrition regimens (2) by the intravenous route are important clinically for many renally impaired and/or burn patients.

Admixtures of fat emulsions with dextrose (3), amino acids (4), and electrolytes (5) are prepared and used for patients as needed. However, it is difficult and complex (6) to mix the fat emulsion with other additives at the time of use. Furthermore, it is necessary to use a large volume of the fat emulsion, (7) as it is continuously administered to the patients intravenously over a long period. If the long-term stability and safety of fat emulsion–parenteral additive mixtures can be corroborated, a more suitable product would result.

The evaluation of emulsion stability using a stepwise progression of procedures was reported by Ho and Higuchi (8). Frank (9) studied the stability of intravenous soybean oil emulsions by gross visual and microscopic examinations. Black and Popovich (10) investigated the effects of such common intravenous additives as physiological electrolytes, amino acid solutions, and/or dextrose on the particle size distribution of the emulsion. These studies, however, failed to characterize the long-term emulsion stability in the presence of the various additives.

In this study, two amino acids were chosen as intravenous additives and were investigated to determine whether or not additives could induce significant changes in the physicochemical properties of an intravenous soybean oil emulsion. The stability of the fat emulsion was evaluated in terms of gross visual observation (11), particle size and distribution of oil droplets measured with a scanning electron microscope (12) and an electronic counter (13), surface tension (14), pH (15), and the zeta potential at the oil–water interface (16). In addition, the effect of the isoelectric point (pI) (17) of the two amino acids and the pH of the buffer solution on emulsion stability were investigated. The study was instituted in an attempt to resolve the conflicts relevant to intravenous fat emulsion compatibility.

EXPERIMENTAL

Preparation of Intravenous Fat Emulsions—The fat emulsion was composed of 100 g of soybean oil (oil phase), 12 g of egg phospholipid (18) (emulsifier), and 25 g of glycerol (to adjust the pH to isotonic) in sufficient distilled water to make 1000 mL. The fatty acid composition of the egg phosphotidyl choline was palmitic acid (28.6%), palmitoleic acid (3.5%), stearic acid (10.6%), oleic acid (37.2%), linoleic acid (11.6%), polyvalent unsaturated fatty acids (6.8%), and other acids (1.7%).

The emulsifier was dissolved in the oil phase, kept at 80°C in a tank, and water preheated to 80°C was added to the solution. The emulsions prepared always contained 10% (w/v) of the oil phase. The agitator used was a mixer[1]; the temperature of the mixture was maintained at 80°C for 30 min after starting agitation in the tank. The impeller speed was kept at 11,200 rpm. The resulting coarse emulsion was quickly introduced into the homogenizer[2] (19) from the mixer and was vigorously stirred again at 4500 psi. Figure 1 illustrates the change of the mean diameter of the oil droplets with shear applications for the emulsions prepared, as measured with an electronic counter[3] (13). The number of shear applications had a large influence on the mean diameter of oil droplets in the emulsion: after eight shear applications, the mean diameter was reduced to ~0.20 μm. The homogenized emulsion was introduced into a glass vessel and was sterilized by heating in a free flame at 121°C for 30 min.

Admixture of the Amino Acids with the Fat Emulsion—In a previous experiment (20), 11 amino acids were selected as intravenous fat emulsion additives. The fat emulsion was admixed with equal parts of a 1% (w/v) amino acid solution. Each of the emulsion combinations was stored at 70°C for 24 h. The height of the separated oil phase appearing in the upper part of emulsion was observed visually at a constant time interval. Table I gives the height of the separated oil phase after admixing with 1% (w/v) amino acid solution. Significant changes in the separated oil phase were found for both the 12- and 24-h readings when L-histidine monohydrochloride monohydrate was admixed with the fat emulsion.

In the present experiments, L-histidine monohydrochloride monohydrate and L-valine were selected as the amino acid additives, because L-histidine monohydrochloride monohydrate has a high isoelectric point (7.64) and L-valine has a low value (5.96). Each amino acid was diluted with each of three solutions, acetate buffer (pH 4.0), phosphate buffer (pH 7.0), and borate buffer (pH 9.0). The amino acid concentration was kept at a constant value of 1% (w/v). The intravenous soybean oil emulsion was admixed with an equal volume of 1% (w/v) amino acid solution, and the mixture was stored at 20°C for 14 d. Fat emulsion admixed with an equal volume of distilled water was used as the control.

Evaluation of Emulsion Stability—The emulsion stability was

[1] Type IM; Tokushukika Co., Ltd., Osaka, Japan.
[2] Type 15M-8TA; Gaulin Co., Ltd., Mass.
[3] Coulter nanosizer; Coulter Electronics Inc., Hialeah, Fla.

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Table I—Height of the Separated Oil Phase of the Fat Emulsion after Adding the Amino Acid Solution[a]

Amino Acid	Height of Oil Phase, %[b] 10 h	12 h	24 h
L-Arginine monohydrochloride	—	—	+
Glycine	—	—	—
L-Histidine monohydrochloride monohydrate	0.67	2.00	2.67
L-Isoleucine	—	—	—
L-Leucine	—	—	—
L-Lysine monohydrochloride	—	—	—
L-Methionine	—	—	—
L-Phenylalanine	—	—	—
L-Threonine	—	—	—
L-Tryptophan	—	—	—
L-Valine	—	—	—

[a] Concentration of amino acid, 1% (w/v); temperature, 70°C; amino acid solution–fat emulsion, 1:1 (v/v). [b] – stable; + unstable.

evaluated by gross visual observation of creaming, particle size distribution, surface tension, pH, and/or zeta potential measurement. These methods have already proven useful in previous work (21). All bottles of the intravenous soybean oil emulsion were refrigerated at 4°C until used.

Gross visual analysis was used to observe separation and creaming of the emulsion after admixing. This analysis utilized five 50-mL glass tubes to display each of the emulsion combinations over the 14-d period. Assessment at predetermined times was qualitative.

The particle size distribution of the emulsions was determined with a scanning electron microscope. A specific fixation technique of the emulsions with malachite green was developed by modifying a previous method (22). Briefly, pieces of filter paper[4] were immersed in the emulsion and rapidly removed. The oil droplets adsorbed on the filter paper were fixed by immersion for 24 h at 4°C in 1% (w/v) glutaraldehyde–malachite green in a buffer solution (NaH_2PO_4–$NaHPO_4$, pH 7.4). After fixation the oil droplets were briefly washed with the buffer solution and allowed to react for 8 h with cold 1% (w/v) osmium tetroxide buffered with phosphate. All samples were subsequently dehydrated in a graded series of ethyl alcohol–water solutions. After critical point drying with liquid carbon dioxide, the fixed oil droplets were mounted on a sample stage with double-sided adhesive tape, vacuum coated with gold, and viewed in the scanning electron microscope[5]. An electronic counter was also used for the determination of the mean diameter of the soybean oil emulsion. The sizing data obtained with the electronic counter were confirmed to be in agreement with those obtained by electron microscopic techniques within an error of 5%.

The surface tensions of the fat emulsions were measured with a Dü-Nouy tensiometer[6]. The hydrogen ion concentration of the sample emulsions was determined using a pH meter[7]. The mean electrophoretic mobility of the oil droplets was obtained with a laser system[8] which measures the average particle mobility in an emulsion; the zeta potential was calculated with these data. All measurements were performed at a thermostatically controlled temperature of 20 ± 0.1°C.

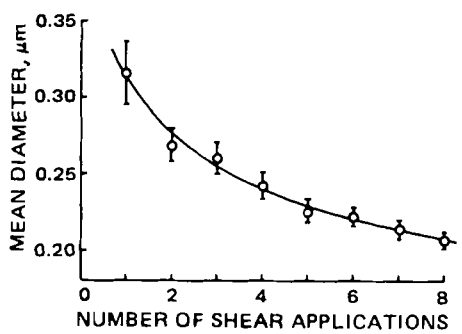


Figure 1—*Changes in the mean diameter of oil droplets in the fat emulsion by application of shear via an homogenizer, as measured by an electronic counter.*

[4] Type 2: Whatman Ltd., England.
[5] Model JSM-T200; JEOL Ltd., Tokyo, Japan.
[6] Shimadzu Seisakusyo Co., Kyoto, Japan.
[7] Model PT-5D; Toyo Kagaku Sangyo Co., Tokyo, Japan.
[8] Laser Zee, model 500; Pen Kem Inc., New York.

Table II—Flocculation Depth of the Fat Emulsion Stored at 20°C After Adding Various Solutions[a]

Solution	Acetate Buffer[b] pH 4.0	Phosphate Buffer[b] pH 7.0	Borate Buffer[b] pH 9.0
1% (v/v) L-Valine	+	+	—
1% (v/v) L-Histidine monohydrochloride monohydrate	1.9	1.5	—
Reference	—	2.9	0.5

[a] Stored temperature, 20°C; data taken 14 days after admixing; adding solution–fat emulsion, 1:1 (v/v). [b] – stable; + large oil droplets appeared on the fat emulsion.

RESULTS

Evaluation of Emulsion Stability in Terms of Separation and Creaming—Table II gives the heights of the separated fat emulsions stored at 20 ± 0.1°C after adding various solutions. In the case where the fat emulsion was admixed with equal parts of phosphate (pH 7.0) or borate (pH 9.0) buffer, separated oil phase was generated in the upper part of fat emulsion. When the fat emulsion was admixed with the borate buffer solution containing 1% (w/v) L-valine, no significant change was detected in the emulsion up to 14 d. However, some large oil droplets appeared on the surface of the fat emulsion when diluted with either acetate or phosphate buffer containing 1% (w/v) L-valine. On the other hand, the emulsion stability remained unchanged when the fat emulsion was diluted with the borate solution containing 1% (w/v) L-histidine monohydrochloride monohydrate. Accordingly, the fat emulsion would be comparatively stable if the admixed solution containing 1% (w/v) amino acid is diluted beforehand with basic buffer solution.

Evaluation of Emulsion Stability by the Particle Size Distribution—Figure 2 shows electron micrographs of oil droplets immediately and 7 d after adding the 1% (w/v) amino acid solutions to the fat emulsions. The amino acid was dissolved in distilled water, and the volume fraction of the fat emulsion to the amino acid solution was 1:1. The electron microscope characterized the fat emulsion as a dispersion of oil globules whose sizes were mostly distributed in a narrow range <1.0 μm.

Particle size distribution curves calculated using the micrographs in Fig. 2 are shown in Fig. 3. For the three samples, the distribution curves and peak heights were similar. A sharp size distribution with a peak at ~0.13 μm was observed. The mean diameter and standard deviation of oil globules were 0.23 μm and 0.11, respectively. The mean diameters as measured by the electronic counter are listed in Table III. No significant difference in the size distribution curves were found between those obtained by the electron microscope and the electronic counter.

Although particle size distributions were not significantly different from each other immediately and 7 d after admixing, a slight change reflected a diminished number of smaller globules (<0.1 μm) relative to the number of large globules (>0.3 μm). This pattern of net globule growth was due to coalescence and is indicative of emulsion instability.

Evaluation of Emulsion Stability in Terms of Surface Tension—Figure 4 shows the changes of surface tension of the fat emulsions with storage time after admixing with 1% (w/v) amino acid solutions. In the case of L-valine, the surface tensions of the fat emulsions diluted with acetate and phosphate buffer solutions decreased markedly at the early

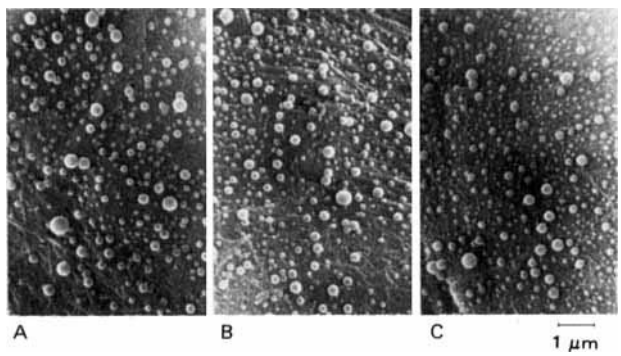


Figure 2—*Scanning electron micrographs of particles in the fat emulsion. The mixture was diluted with distilled water; the amino acid solution–fat emulsion ratio was 1:1 (v/v). Key: (A) L-valine; (B) reference solution; (C) L-histidine monohydrochloride monohydrate.*

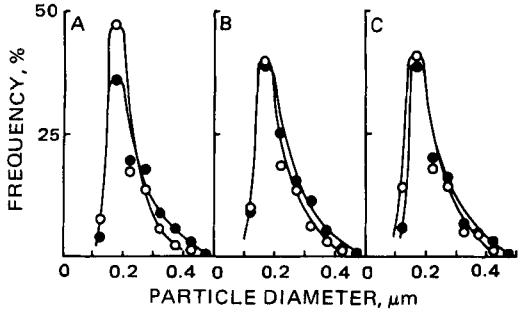


Figure 3—*Particle size distribution in the fat emulsion immediately (○) and 7 d (●) after adding a 1% (w/v) solution of amino acid in distilled water. The amino acid–fat emulsion ratio was 1:1 (v/v); the prepared final fat emulsion was stored at 20°C. Key: (A) L-valine; (B) reference solution; (C) L-histidine monohydrochloride monohydrate.*

stage of storage, and then leveled off after 4 d. However, the surface tension of the fat emulsion in borate buffer solution decreased slowly with increase in storage time.

In the case of L-histidine monohydrochloride monohydrate, the surface tension of the fat emulsion diluted with acetate buffer solution lowered suddenly to 33 dyn/cm after admixing. However, the surface tension of the fat emulsion containing L-histidine monohydrochloride monohydrate had the same tendency to decrease in both phosphate and borate buffer solutions.

A decrease of surface tension of an emulsion may be interpreted as showing that a certain number of large oil globules appear and move to the surface layer of the emulsion. Accordingly, the fat emulsions would be comparatively stable if the admixed solution containing 1% (w/v) amino acid is previously diluted with the basic buffer solution.

Evaluation of Emulsion Stability in Terms of pH—The effects of adding 1% (w/v) amino acid solutions on the pH of the fat emulsions are shown in Fig. 5. Addition of a 1% (w/v) solution of L-valine or L-histidine monohydrochloride monohydrate did not appreciably alter the pH of the fat emulsion in any of the buffer solutions. However, amino acid solutions immediately altered the pH of the fat emulsion when distilled water was used instead of the buffer solution. Thus, one should avoid the use of distilled water alone as a diluent when a fat emulsion is admixed with solutions of L-valine or L-histidine monohydrochloride monohydrate.

Evaluation of Emulsion Stability in Terms of Zeta Potential—Zeta potential is a controlling parameter in determining the stability of an emulsion. Figure 6 shows the change with time of the zeta potential of oil droplets in the fat emulsion containing 1% (w/v) amino acid. At the initial stage of admixing, the zeta potential ranged between −30 and −60 mV. Thus, the fat emulsion is likely to be sufficiently stable even though the amino acid is admixed because the strong electrostatic repulsion prevents two oil droplets from coalescing.

The zeta potential readings revealed that the addition of the amino acid solution altered the zeta potential of oil droplets within 1 d. However, after this initial change, the zeta potential remained constant throughout the 14-d observation period. This indicates that the amino acids changed the electrical properties of the oil–water interface very quickly at the early stage of admixing, and the zeta potential of the interface later shifted slightly as the storage time was increased.

DISCUSSION

When the fat emulsion admixed with 1% (w/v) L-histidine monohydrochloride monohydrate was stored at 70°C over a 24-h period, there was a significant change in the height of the separated oil phase. However,

Table III—Mean Diameter of Droplets in the Fat Emulsion [a]

Solution	Mean Diameter, μm: Scanning Electron Microscope, Day 0	Scanning Electron Microscope, Day 7	Electronic Counter, Day 0	Electronic Counter, Day 7
L-Valine	0.213	0.242	0.215	0.238
L-Histidine monohydrochloride monohydrate	0.211	0.230	0.213	0.235
Reference	0.214	0.227	0.212	0.224

[a] Mixture was diluted with distilled water; amino acid solution–fat emulsion, 1:1 (v/v); fat emulsion was stored at 20°C.

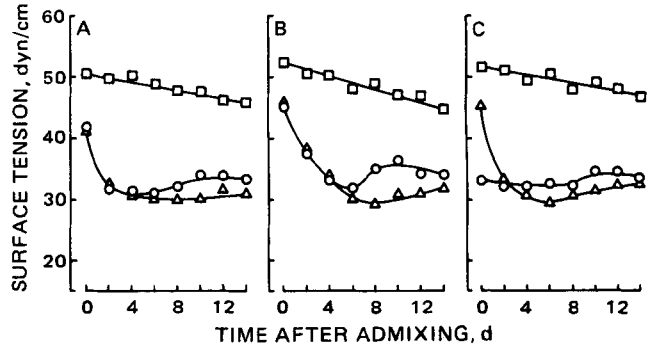


Figure 4—*Effect of adding 1% (w/v) solutions of L-valine (A), the reference (B), and L-histidine monohydrochloride monohydrate (C) on the surface tension of the fat emulsion. The amino acid solution–fat emulsion ratio was 1:1 (v/v); the prepared final fat emulsion was stored at 20°C. Key: (□) borate buffer, pH 9.0; (△) phosphate buffer, pH 7.0; (○) acetate buffer, pH 4.0.*

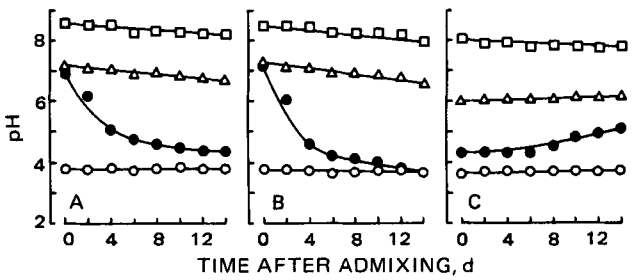


Figure 5—*Effect of adding 1% (w/v) solutions of L-valine (A), the reference (B), and L-histidine monohydrochloride monohydrate (C) on the pH of the fat emulsion. The amino acid solution–fat emulsion ratio was 1:1 (v/v); the prepared final fat emulsion was stored at 20°C. Key: (□) borate buffer, pH 9.0; (△) phosphate buffer, pH 7.0; (○) acetate buffer, pH 4.0; (●) distilled water.*

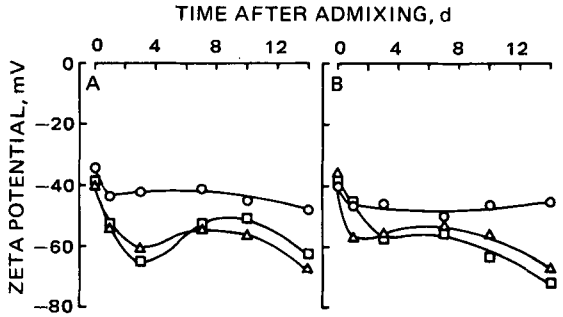


Figure 6—*Change with time of the zeta potential of oil droplets in the fat emulsion containing 1% (w/v) L-valine (A) or L-histidine monohydrochloride monohydrate (B). The amino acid solution–fat emulsion ratio was 1:1 (v/v); the prepared final fat emulsion was stored at 20°C. Key: (□) borate buffer, pH 9.0; (△) phosphate buffer, pH 7.0; (○) acetate buffer, pH 4.0.*

if the fat emulsion was stored at 20°C, the separated oil phase appeared gradually ~10 d after admixing. Hence, the rate of separation of the oil phase in the fat emulsion was considered to be very slow if the fat emulsion was stored at room temperature after being mixed with the 1% (w/v) amino acid solution.

The particle size distribution of the fat emulsion containing 1% (w/v) amino acid shifted only slightly to a larger size after a 7-d period. Accordingly, one may assume that coalescence or creaming of oil droplets rarely occurs in the fat emulsion.

The surface tension of the fat emulsion admixed with 1% (w/v) amino acid solution decreased markedly at the early stage of storage. Hence, it was considered that a certain number of large oil globules appeared and were concentrated in the surface layer of the fat emulsion.

No significant pH change was found when the fat emulsion was diluted with buffer solution instead of distilled water. Thus, buffered amino acid solutions only slightly affected the pH of the fat emulsion.

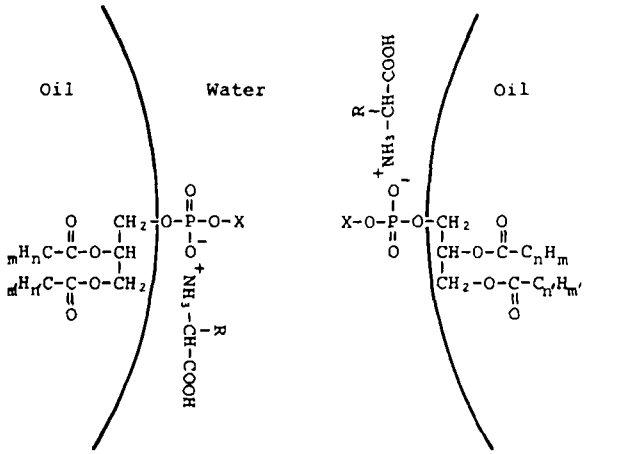
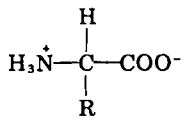


Figure 7—*Schematic representation of electrolytic dissociation of phospholipid and amino acid molecules adsorbed at the oil–water interface in acidic solution.*

At the initial stage of storage after admixing with 1% (w/v) amino acid solution, the absolute value of the zeta potential of the fat emulsion increased, and this increase was more frequently observed with the basic buffer solution than with the acidic buffer solutions. Therefore, it may be considered that amino acid in a basic buffer solution produced a strong electrostatic repulsion force at the surface of the oil droplets. These evaluations of emulsion stability concluded that the fat emulsion may be sufficiently stable even 14 d after adding 1% (w/v) amino acid if stored at 4°C.

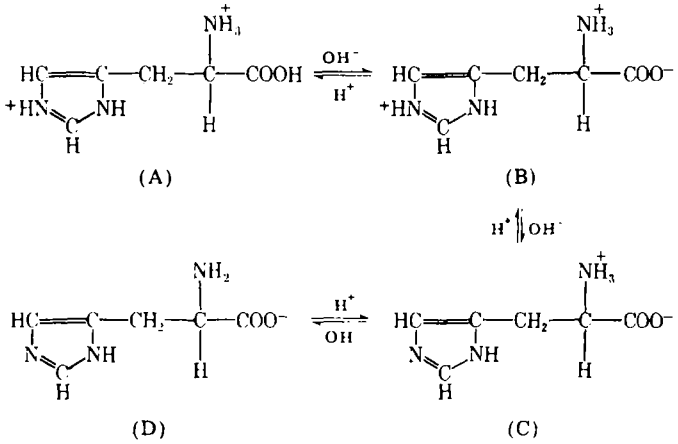
Amino acids in aqueous solution exist generally as dipolar ions:



As the isoelectric point of L-valine is 5.96, it exists in acetate buffer solution (CH_3COOH–CH_3COONa, pH 4.0) as a positively charged ion. That is, L-valine as a positively charged ion in pH 4.0 buffer solution is adsorbed on the negatively charged oil droplet surface. As shown in Fig. 7, the charge will be neutralized. On the other hand, when hydroxyl ions are added to the amino acid solution, dissociation of the carboxyl group of the amino acid will take place.

L-Valine exists in pH 7.0 and pH 9.0 buffered solutions as a negatively charged ion. Accordingly, negatively charged oil droplets and amino acid molecules (L-valine molecules) will repel each other in the above buffer solutions. Figure 8 shows a schematic representation of electrolytic dissociation of phospholipid and amino acid molecules adsorbed at the oil–water interface in basic solution. In this state, oil droplets in the fat emulsion are electrically stabilized.

The dissociation of L-histidine monohydrochloride monohydrate in acidic or basic solution is illustrated in Scheme I.



Scheme I

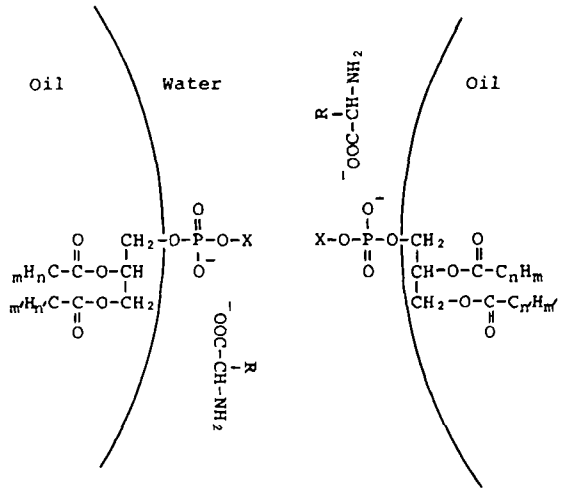


Figure 8—*Schematic representation of electrolytic dissociation of phospholipid and amino acid molecules adsorbed at the oil–water interface in basic solution.*

Since the pK_a value of the imidazole nitrogen is lower than that of the ammonium group, a proton on the imidazole nitrogen in weakly basic solution is removed; when hydroxyl ions are added, a proton on the ammonium group is removed. L-Histidine monohydrochloride monohydrate in pH 9.0 buffer solution is in the form of anion since the isoelectric point (pI) of L-histidine monohydrochloride monohydrate is 7.59. That is, negatively charged oil droplets and L-histidine monohydrochloride monohydrate molecules repel each other in this buffer solution. Thus, the fat emulsion is considered to be stable in basic solution. Accordingly, it will be necessary to take into account the effects of the isoelectric point of the amino acid and the hydrogen ion concentration of buffer solutions on the emulsion stability of intravenous fat emulsions.

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