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Report

Physicochemical Characterization of Parenteral Lipid Emulsion: Determination of Hamaker Constants and Activation Energy of Coalescence

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The particle size of lipid emulsion (LE) is changed by flocculation and coalescence. This change in particle size was studied using values obtained for maximum total interaction energy (Vt^{max}) for flocculation and activation energy for coalescence (E). Vt^{max} was calculated using DLVO theory, and E was calculated from the rate of increase in particle size in LE. Two LEs (PC99LE and PC70LE) were prepared from lecithins containing 99% and 70% phosphatidylcholine, respectively. The Hamaker constants for PC99LE and PC70LE were found to be 1.4×10^{-22} J and 3.1×10^{-21} J, respectively. Vt^{max} for PC99LE was 4.7 kT at 121°C, and E was 1.5×10^{-19} J, while Vt^{max} for PC70LE at 121°C was 151 kT and E was 3.2×10^{-19} J. These findings suggest that PC99LE readily underwent flocculation and coalescence with increase in particle size, but that the particle size of PC70LE changed little. The degrees of flocculation and coalescence of LE were determined separately using values of Vt^{max} and E. These parameters are thus quite useful in predicting the stability of LE.

KEY WORDS: emulsion; flocculation; coalescence; DLVO theory; phosphatidylcholine; Hamaker constant.

INTRODUCTION

Parenteral lipid emulsions (LE) have been commonly used as nutrients (1). Recently, LE containing active drugs in the oil phase have been used as drug carriers in drug delivery systems (2–4).

It is well known that the stability and the behavior of LE in the body are related to the surfactant used and the condition of emulsification (5). However, few thermodynamic studies have been made of change in size (6), flocculation or coalescence of lipid particles. It is important that LE particle size be controlled at the time of preparation, since it is increased by the steam under pressure sterilization (SUPS) process.

We therefore used DLVO theory (7,8) to study the process of flocculation of LE. However, the process of increase in particle size cannot be understood with this theory alone, since particle destruction also occurs. Thus, flocculation and coalescence of LE must be considered separately. It appears to be the case that the flocculation process can be analyzed with DLVO theory in a fashion similar to that for flocculation of colloid. Initially, the Hamaker constant, which characterizes the magnitude of van der Waals attractive forces acting between colloidal particles, must be determined for analysis using DLVO theory. The Hamaker constant for liposomes was reported by Ohshima *et al.* (9) to be approximately 4×10^{-21} J. However, LE differs from liposomes, since it contains oil particles and has a single lamella interface. Nevertheless, the Hamaker constant can be determined from the critical flocculation concentration (*c.f.c.*) using a method reported by C. Washington *et al.* (10). It should therefore be possible to study the process of flocculation of LE with DLVO theory. On the other hand, the total energy of the increase in particle size is equal to the sum of the energy of flocculation and coalescence. We analyzed the stabilities of LE prepared with two kinds of lecithins (PC99, PC70) using this method. PC99 and PC70 are lecithins containing 99% and 70% phosphatidylcholine (PC), respectively. They have been used in drug delivery systems and for nutrients. Only PC99 and PC70 can be used for surfactants in commercial parenteral lipid emulsions. We found that LE containing PC99 was differ from LE containing PC70 in stability of emulsion. Purpose of this work makes scientifically clear the mechanism of flocculation and coalescence process of LE.

THEORETICAL

Total Interaction Energy (Vt) on Flocculation of LE

Vt is the sum of contributions of the potential energy of electrostatic interaction V_R and that of the van der Waals interaction V_A:

$$Vt(H) = V_R(H) + V_A(H). \qquad (1)$$

For bulk solutions containing two kinds of electrolytes, we derived an expression for V_R between two identical spheres immersed in a mixed solution of 1:1 and 2:1 electrolytes using the DLVO theory of colloid stability (7,8) as follows.

Consider first the electric potential Ψ produced by a single plate with a surface potential Ψ_0 immersed in a mixed solution of 1:1 and 2:1 electrolytes. Let n_1 and n_2, respectively, be the number densities (in units of m^{-3}) of 1:1 and 2:1 electrolytes in the bulk solution phase. Let the x-axis be perpendicular to the plate with its origin 0 at the plate surface so that the region $x > 0$ is the solution phase. The Poisson-Boltzmann equation for the electric potential $\Psi(x)$ at position x relative to the bulk solution phase, where $\Psi(x)$ is set equal to zero, is then given by (11)

$$\frac{d^2\Psi}{dx^2} = -\frac{e}{\epsilon_r\epsilon_0}\left[n_1 \exp\left(-\frac{e\Psi}{kT}\right) + 2n_2 \exp\left(-\frac{2e\Psi}{kT}\right) - (n_1 + 2n_2)\exp\left(\frac{e\Psi}{kT}\right)\right], \qquad (2)$$

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NOTATIONS: LE, lipid emulsion; SUPS, steam under pressure sterilization; Vt^{max}, maximum total interaction energy; E, activation energy for coalescence; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PC99, 99% PC; PC70, 70% PC; *c.f.c.*, critical flocculation concentration.

where e is the electric unit charge, ϵ_r is the relative permittivity of solution, ϵ_0 is the permittivity of vacuum, k is the Boltzmann constant, and T is the absolute temperature. Eq. 2 can easily be integrated to obtain

$$\frac{[(1-\eta/3)\exp(y)+\eta/3]^{1/2}-1}{[(1-\eta/3)\exp(y)+\eta/3]^{1/2}+1} = \frac{[(1-\eta/3)\exp(y_0)+\eta/3]^{1/2}-1}{[(1-\eta/3)\exp(y_0)+\eta/3]^{1/2}+1}\exp(-\kappa x), \qquad (3)$$

with

$$y = \frac{e\Psi}{kT}, \qquad y_0 = \frac{e\Psi_0}{kT},$$

$$\eta = \frac{3n_2}{n_1+3n_2}, \qquad \kappa = \left[\frac{2(n_1+3n_2)e^2}{\epsilon_r\epsilon_0 kT}\right]^{1/2}, \qquad (4)$$

where y is the scaled potential, y_0 is the scaled surface potential, and k is the Debye-Hückel parameter. If the left-hand side of Eq. 3 is expanded with respect to y, we obtain, by retaining only the term proportional to y,

$$y(x) = 4\gamma\exp(-\kappa x), \qquad (5)$$

with

$$\gamma = \frac{1}{(1-\eta/3)}\frac{[(1-\eta/3)\exp(y_0)+\eta/3]^{1/2}-1}{[(1-\eta/3)\exp(y_0)+\eta/3]^{1/2}+1}, \qquad (6)$$

Eq. 5 will be used later.

Consider next the electrostatic interaction between two similar plates with a surface potential Ψ_0 separated by a distance h between their surfaces in a mixed solution of 1:1 and 2:1 electrolytes. According to the DLVO theory of colloid stability, the repulsive pressure $P(h)$ acting between the two plates is given by

$$P(h) = [n_1\exp(-y_m) + n_2\exp(-2y_m) + (n_1 + 2n_2)\exp(y_m) - (2n_1 + 3n_2)]kT, \qquad (7)$$

where y_m is the value of the reduced electric potential at the midpoint between the plates. Using the approximation employed in DLVO theory, we assume that $|y_m| \ll 1$. In this case, Eq. 7 reduces to

$$P(h) = (n_1 + 3n_2)kTy_m^2. \qquad (8)$$

In accordance with the assumption that $|y_m| \ll 1$, we can also assume that y_m is given by the sum of the unperturbed potentials produced by the respective plates at position $x = h/2$ in the absence of interaction, *viz.*,

$$y_m = 2y(h/2), \qquad (9)$$

where $y(h/2)$ can be obtained from Eq. 5 for the electric potential y for a single plate. By combining Eqs. 5, 8, and 9, we obtain

$$P(h) = 64(n_1 + 3n_2)\gamma^2kT\exp(-\kappa h). \qquad (10)$$

The potential energy of the electrostatic interaction $V_{pl}(h)$ between the two plates per unit area is thus given by

$$V_{pl}(h) = \int_h^\infty P(h)dh = \frac{64(n_1 + 3n_2)\gamma^2kT}{\kappa}\exp(-\kappa h). \qquad (11)$$

Now consider the potential energy $V_R(H)$ of the electrostatic interaction between two identical spherical particles of radius a at separation H between their surfaces. With the help of Derjaguin's (12) approximation, we can obtain an expression for $V_R(H)$ from the corresponding expression for the interaction between two parallel plates, viz.,

$$V_R(H) = \pi a\int_H^\infty V_{pl}(h)dh = \frac{64\pi a(n_1 + 3n_2)\gamma^2kT}{\kappa^2}\exp(-\kappa H). \qquad (12)$$

Note that for the case of symmetrical electrolytes ($n_2 = 0$), Eq. 12 reduces to an expression for the interaction energy derived in DLVO theory.

V_A can be calculated as:

$$V_A = -\frac{Aa}{12H}, \qquad (13)$$

where A is the Hamaker constant.

Thus, from Eq. 12 and Eq. 13, Eq. 1 can be derived. Two LE particles are flocculated when the maximum in the potential curve $V_t = V_R + V_A$, *i.e.*, the potential barrier, disappears (see Eq. 15).

Activation Energy for Coalescence (E)

It has been reported (13) that the following equation can be used to predict the rate of increase in LE particle volume:

$$\frac{dv}{dt} = \frac{4kT}{3\eta}\phi\exp(-E/RT), \qquad (14)$$

where v is the mean volume of a particle, η the viscosity of the bulk solution, ϕ the volume fraction of bulk solution, and R the gas constant. Therefore the activation energy, E, which includes the energy for destruction of the interfacial membrane in addition to the maximum total interaction energy (Vt^{max}) as the energy barrier, can be obtained from the plot of the rate of increase in particle volume.

MATERIALS AND METHODS

Materials

PC99 and PC70 were purchased from Asahikasei Co., Ltd. Soybean oil was obtained from Ajinomoto, Co., Inc., and concentrated glycerin from Kozakai Pharmaceutical Co., Ltd. All other chemicals were of reagent grade and from Wako Pure Chemical Industries, Ltd.

Preparation of LE

One thousand ml of LE (PC99LE, PC70LE) were prepared using the method described in our previous report (14) with 18 g of PC99 or PC70, 100 g of soybean oil and 22.5 g of glycerol to achieve isotonicity.

Measurements

The mean particle size of LE was measured using a laser light scattering spectrometer (Nicomp Model 370 Submicron Particle Sizer, Pacific Scientific Instrument Division). The pH of LE was measured with a pH meter at room temperature (Model HM-30, Toa Denpa Kogyo Co., Ltd.). The ζ potential was calculated from the mean particle electrophoretic mobility. Measurements of mean electrophoretic mobility were made in the same solution containing 0.5 mM potassium chloride (Model DELSA 440, Coulter Electronics, Ltd.).

Study of Influence of pH and Temperature on ζ Potential

LE was diluted 4000 times with aqueous potassium chloride solution (ionic strength 0.0005), which had been adjusted to various pHs using HCl or KOH. The mean electrophoretic mobility of each solution was measured at 25°C, and the ζ potential was calculated.

LE was diluted with aqueous solution of pH 4 or 8, and the mean electrophoretic mobility was similarly measured at 10, 25, and 60°C.

Determination of Hamaker Constants

LE was diluted 200 times with 0.01 M KCl aqueous solution, and stirred at 25°C. Various concentrations of $CaCl_2$ aqueous solution were added to each mixture, and the absorbance of each mixture was measured over time at 420 nm. The initial rate of change in absorbance was calculated by the method of least squares using the initial three values of that, and the *c.f.c.* of $CaCl_2$ was obtained. However, the Vt^{max} for stable colloid generally is large enough to prevent flocculation. If electrolyte is added to this colloidal solution, Vt^{max} decreases, and the colloid flocculates. The *c.f.c.* is obtained as the concentration of electrolyte at the beginning of flocculation. The following requirements must be met:

$$Vt(H) = 0, \quad \frac{dV(H)}{dH} = 0. \tag{15}$$

Eq. 15 corresponds to the condition of "fast" flocculation. Thus, using Eq. 15, Eq. 1 can be solved, and it is found that

$$3.85 \times 10^{-39} \times \frac{\gamma^4}{A^2} = c.f.c. \tag{16}$$

Here, *c.f.c.* can be obtained as:

$$c.f.c. = C_1 + 3C_2, \tag{17}$$

where C_1 and C_2 are concentrations of 1:1 and 2:1 electrolytes, respectively. By substituting values of C_1 and C_2, which are obtained experimentally, into Eqs. 16 and 17, the Hamaker constant can be obtained.

Storage Test at High Temperature

LE-filled ampoules were immersed in an oil bath, and the temperature was maintained at 100, 125, 150, 165 or 180°C. Samples were removed over time, and their pH and mean particle size were measured. The manufacturing process for injections generally included SUPS. Therefore, the stability test was performed using SUPS at 121°C for 20 minutes.

Table I. Properties of LEs

	PC99LE	PC70LE
pH	6.5	6.1
Mean particle size (nm)	204	208
ζ potential (mV)[a]	−8	−46

[a] Each value was obtained from measurement of the mean particle electrophoretic mobility in 0.5 mM KCl solution at the same pH of LE.

RESULTS AND DISCUSSION

Physicochemical Properties

pHs, mean particle sizes and ζ potentials for PC99LE and PC70LE are shown in Table I. The pHs and mean particle sizes of PC99LE and PC70LE were the same, but their ζ potentials differed. The LE pH-ζ potential profiles are shown in Fig. 1. In addition, ζ potentials obtained from Fig. 1 by interpolation are listed in Table I. The absolute ζ potentials of both LEs increased with increasing pH. Since variation in pH affected the charge on lecithin, it appeared that the negative charge on the lipid particle surface became larger with increasing pH. The absolute ζ potential of PC70LE was larger than that of PC99LE. Ishii *et al.* reported (15) that the absolute ζ potential of LE composed of phosphatidylethanolamine (PE) was larger than that of LE composed of PC. The absolute ζ potential thus appeared to influence PE content of egg yolk lecithin, which comprises the interfacial membrane.

Determination of the Hamaker Constants

It was assumed that the initial rate of change in absorbance appeared its flocculation rate (16). Each of the relationships between concentration of $CaCl_2$ and rate of change in absorbance so obtained are plotted in Fig. 2. Each of the concentrations of $CaCl_2$ on the corners of the curves was obtained from Fig. 2 as values of C_2 for calculation of c.f.c. The values of C_2 used to calculate *c.f.c.* for PC99LE and PC70LE were 0.4 mM and 1.16 mM, respectively. In addition, the relationships between concentration of $CaCl_2$ and ζ potential are illustrated in Fig. 3. As shown, the ζ potentials

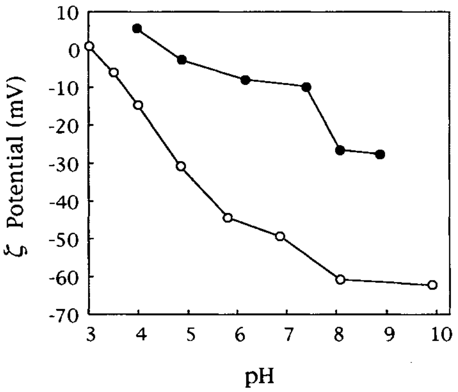

Fig. 1. ζ potentials of PC99LE (●) and PC70LE (○) in 0.5 mM KCl solution as a function of pH at 25°C. pHs of LEs were adjusted by 0.1 N HCl or 0.1 N KOH. (n = 2).

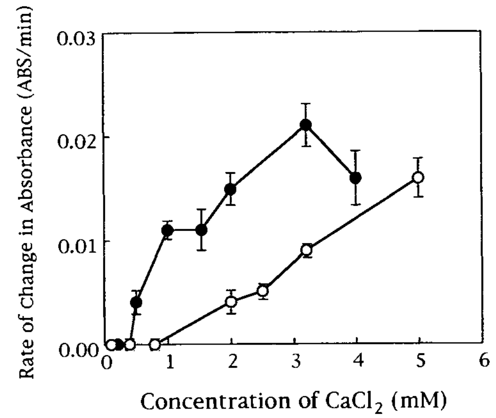

Fig. 2. The rates of change in absorbance of PC99LE (●) and PC70LE (○) as a function of CaCl$_2$ concentration with 0.01 M KCl at 25°C. Plots and error-bars show means ± SD for three times run.

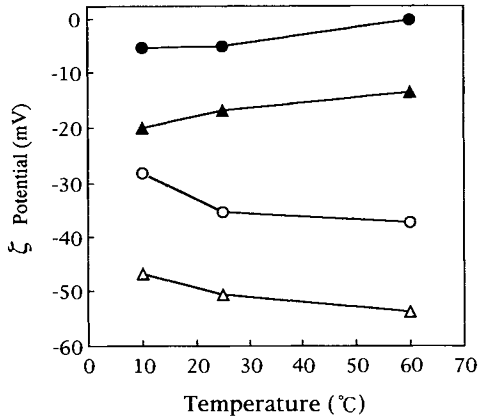

Fig. 4. ζ potentials of PC99LE at pH 4 (●) and pH 8 (○), and of PC70LE at pH 4 (▲) and pH 8 (△), in 0.5 mM KCl solution as a function of temperature.

for PC99LE and PC70LE at *c.f.c.* were −1.6 mV and −8 mV, respectively. The Hamaker constants were calculated by substitution of these values in Eqs. 16 and 17. The values so obtained for PC99LE and PC70LE were 1.4×10^{-22} and 3.1×10^{-18}, respectively. These indicate that PC99LE flocculated more easily than PC70LE despite its smaller Hamaker constant. This may have occurred because the stability of the particles is strongly influenced by the ζ potentials.

Influence of SUPS on Mean Particle Size

PC99LE and PC70LE were sterilized by SUPS. SUPS did not change the pH of PC99LE, but did increase its mean particle size to 320 nm. On the other hand, neither the pH nor mean particle size of PC70LE was affected by SUPS.

Values of Vt^{max} at 121°C were calculated for LE using Eq. 1 by substitution of ζ potentials, mean particle sizes and the Hamaker constants as above. The relationship between ζ potential and temperature was determined in a preliminary study. The ζ potential of LE was measured over a range of temperatures, as shown in Fig. 4. Since the ζ potential of LE was little affected by temperature, the ζ potential at 25° was used to calculate of Vt^{max} at other temperatures. The values of Vt^{max} for PC99LE and PC70LE at 121°C obtained in this fashion were 4.7 kT and 151 kT, respectively. Since this value of Vt^{max} for PC99LE was quite small, PC99LE appeared to undergo flocculation readily. On the other hand, since the value of Vt^{max} for PC70LE was very large, its energy for flocculation was large and it did not readily undergo flocculation.

The effect of pH on change in particle size was studied using SUPS. PC99LE and PC70LE were adjusted to various pHs with 0.1 N KOH or 0.1 N HCl, and the ratios of changes in mean particle sizes are shown in Fig. 5. The ratio of changes in mean particle sizes, R, was obtained as:

$$R = \frac{\text{mean particle size after SUPS}}{\text{mean particle size before SUPS}}.$$

The value of R for PC99LE increased as pH decreased beneath pH 8.0, but mean particle size changed little at values of pH above 8.0. On the other hand, the value of R for PC70LE was approximately 1 at values of pH above 4.0, but mean particle sizes increased very little at pH 4.0 or below. The ζ potentials of these LEs before SUPS were obtained from Fig. 1 by interpolating to pH of the LE, and values of Vt^{max} at 121°C were calculated from Eq. 1. Using these values, the relationships between R and Vt^{max} for PC99LE and PC70LE were determined, and are illustrated in Fig. 6. Both R values increased as Vt^{max} decreased, if Vt^{max} was less than 15 kT. The mean particle sizes of PC99LE and PC70LE were undergone since values of Vt^{max} were below 15 kT. These findings indicated that both PC99LE and PC70LE flocculated easily if their Vt^{max} values was less than 15 kT. This is the "slow" flocculation, which differs from the

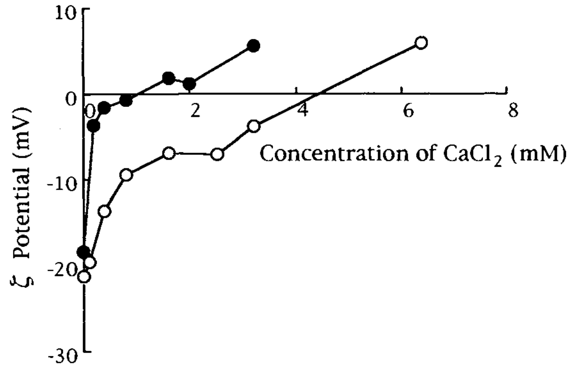

Fig. 3. ζ potentials of PC99LE (●) and PC70LE (○) as a function of CaCl$_2$ concentration with 0.01 N KCl at 25°C.

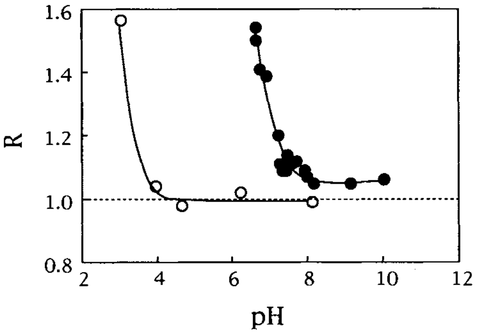

Fig. 5. The ratios of change in mean particle size before and after SUPS (R) of PC99LE (●) and PC70LE (○) as a function of pH.

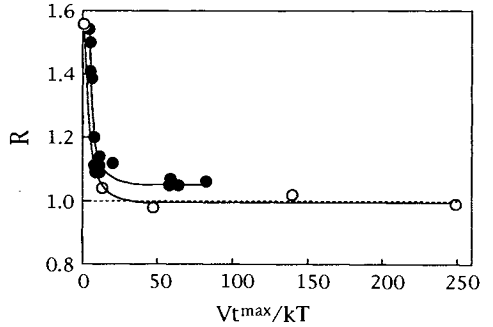

Fig. 6. The ratios of change in mean particle size before and after SUPS (R) of PC99LE (●) and PC70LE (○) as a function of Vt^{max}. Vt^{max} was calculated with T = 394 K.

"fast" flocculation occurred in the absence of the potential barrier. Values of Vt^{max} above 15 kT were obtained above pH 8.0 and above pH 4.0 for PC99LE and PC70LE, respectively. These findings demonstrated that the stability of various LEs would be evaluated using our method.

Influence of Temperature on Stability of LE

The mean particle size of PC99LE (pH 6.5) increased with time at certain temperatures (100, 125, 150°C). Mean particle volumes were calculated from mean particle sizes, and the results shown in Fig. 7. Mean particle volumes increased linearly over time until 10 min. Those of PC70LE (pH 6.1) are shown in Fig. 8. These initial mean particle volume changes did not appear at all temperatures. These findings indicated that the mean particle volume of PC70LE increased from appropriate intervals, and that those intervals grew shorter as temperature increased.

The initial rates of increase in mean particle volume of PC99LE and PC70LE were obtained by approximation to zero-order kinetics. The relationships between initial rate of increase and the reciprocal of the absolute temperature were linear, and are illustrated in Fig. 9. Using the slopes of these lines, values of E for PC99LE and PC70LE were calculated with Eq. 14, and are listed in Table II. Increase in particle volume has two processes which are flocculation and coalescence. It therefore is considered that activation energy obtained relates both flocculation and coalescence. Increase in the particle volume occurs by outside energy which is larger than sum of Vt^{max} and energy for coalescence. The value of E for PC70LE was more than twice as large as that for PC99LE. PC70LE was more resistant to undergoing flocculation or coalescence than PC99LE. The mean particle volume of PC99LE increased rapidly at high temperatures, while that of PC70LE increased after a time lag. Since increase in the particle size of PC70LE began after pH reached 5.1, time lags in reaction of PC70LE appeared at the times of pH change. The Vt^{max} for PC70LE at 150°C was 142 kT at pH 6.1, and thus large. Therefore, PC70LE was not expected to flocculate, and its particle size did not increase immediately, but its ζ potential became small as pH decreased. For this reason, Vt^{max} became small and PC70LE flocculated easily after the time lag. It appeared that the decrease in pH was the reason for oxidization of the lecithin, and that the increase in mean particle size was due to ease of flocculation.

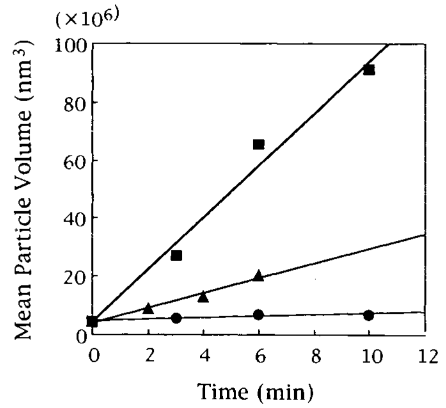

Fig. 7. The mean particle volumes of PC99LE in storage test for various periods of time at 100 (●), 125 (▲) and 150 (■) °C.

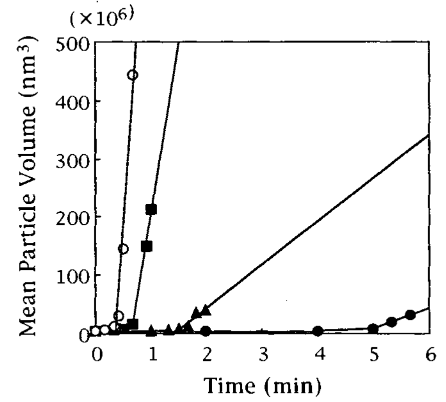

Fig. 8. The mean particle volumes of PC70LE in storage test for various periods of time at 150 (●), 165 (▲), 175 (■) and 180 (○) °C.

PE has an ethanolamine substituent, while PC has a choline one. The positive electric charge of the quaternary amine in PE is smaller than that of the monoamine in PC. Therefore, the negative charge of PC70LE containing 17% PE is larger than that of PC99LE.

CONCLUSIONS

We determined the Hamaker constants of two LEs from critical flocculation concentrations. They were similar in value to those of liposomes reported by Ohshima *et al.*

The Vt^{max} for PC99LE was smaller than that for

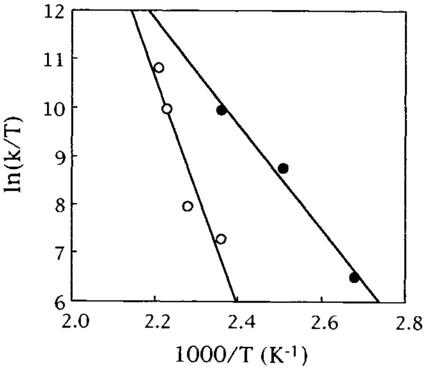

Fig. 9. Plots of ln (k/T) *versus* 1000/T: PC99LE (●); PC70LE (○).

Table II. Activation Energies for Coalescence (E) of LEs

	PC99LE	PC70LE
E (kJ/mol)[a]	90.2	195
(J)[b]	1.50×10^{-19}	3.23×10^{-19}

[a] Each value was calculated by Eq. 17.

[b] Each value was divided value of [a] by Avogadro's number.

PC70LE. Thus, PC70LE was not readily flocculated, nor was its particle size increased, by SUPS. The stability of LE was affected by the purity of the lecithin. It appeared that the tendency of LE to undergo flocculation and coalescence should be evaluated using values of Vt^{max} and E. PC99LE flocculated more easily than PC70LE despite its smaller Hamaker constant. This may have occurred because of the strong influence of ζ potentials on particle stability. The Vt^{max} and the E are quite useful in predicting whether the particle size of parenteral lipid emulsion will increase.

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