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Physicochemical Characterization of Parenteral Lipid Emulsion: Influence of Cosurfactants on Flocculation and Coalescence

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Purpose. The stability of lipid emulsions (LE) containing various cosurfactants (oleic acid, cholesterol, Tween 80, or HCO-60) was evaluated using the maximum total interaction energy, Vt^{max}, and the energy barrier for coalescence, W. ***Methods.*** The Vt^{max} and W were calculated from the ζ potential and the rate of increase in LE particle size, respectively. ***Results.*** The Vt^{max} and W of LE containing the oleic acid were 0.598×10^{-19} J and 3.03×10^{-19} J, respectively, while those of LE without the cosurfactant were 0.141×10^{-19} J and 1.36×10^{-19} J. ***Conclusions.*** These findings suggest that oleic acid prevents the flocculation and coalescence of LE. The Vt^{max} and W of LE containing the cholesterol were 0.435×10^{-19} J and 0.63×10^{-19} J, respectively, suggesting that the cholesterol prevents the flocculation of LE but does not affect the coalescence. Analysis of the stability of LE was performed by the separate considerations of the flocculation and coalescence.

KEY WORDS: lipid emulsion; flocculation; coalescence; DLVO theory; phosphatidylcholine.

INTRODUCTION

Parenteral lipid emulsions (LE) are presently being used as nutrients. Recently, LEs have come to be used as drug carriers in drug delivery systems (1). For example, prostaglandin E_1 (PGE_1) and clinprost (an isocarbacyclin derivative) in an injectable LE can be retained in lipid particles. These preparations are referred to as Lipo-PGE_1 (2) and Lipo-PGI_2 (3). Both display remarkably high activity in clinical treatment. Emulsion stability is a property of great importance in these LE, and many studies regarding this stability have been reported (4). Emulsion stabilization is studied using cosurfactants for cream, milky lotion, and ointment. However, as for parenteral LE, an egg yolk lecithin (PC), which possesses poor emulsification capability, is commonly used. The effects of cosurfactant on emulsification capability have not been extensively investigated, despite their importance. Washington and Davis (5) reported that the surface negative electric potential of LE was decreased by the addition of a fatty acid such as oleic acid. Emulsion stability has usually been evaluated by flocculation rate, or separation using interfacial tension (6), and often by surface electric potential alone (7,8). However, these methods are time consuming and may not reflect actual behavior. On the other hand, it is well established that the stability and behavior of LE in the body are related to the surfactant used and the condition of emulsification. However, few thermodynamic studies have been made of change in size, flocculation, or coalescence of lipid particles in LEs. We therefore studied the change in particle size during steam under pressure sterilization (SUPS) by considering flocculation and coalescence separately. In our previous study (9), the stabilities of two kinds of LE (PC99LE and PC70LE) prepared from PC containing 99% and 70% phosphatidylcholine were determined and the effect of the maximum total interaction energy (Vt^{max}) on the flocculation was clarified.

In general, DLVO theory is useful for analysis of colloid flocculation (10,11). DLVO theory makes use of the potential energy of electrostatic interaction and that of the van der Waals interaction between two particles of the same kind. Lawrence and Mills (12) measured changes in particle volume at several temperatures, and analyzed thermodynamically the coalescence which occurred after flocculation. In the present study, the effect of adjuvants on emulsion stability was evaluated using the Vt^{max} and appearance activation energy *(E)* of coalescence obtained from the rate of increase in particle size. The adjuvants used were oleic acid and cholesterol which had been used as cosurfactants. Other adjuvants used were sorbitan monooleate (Tween 80) and hydrogenated castor oil (HCO-60) which had been used as surfactants. We used these adjuvants as cosurfactants for emulsification using egg yolk lecithin.

MATERIALS AND METHODS

Materials

Purified PC, which contains 99% phosphatidylcholine (PC99), was purchased from Asahikasei Co. (Tokyo, Japan). Purified soybean oil was purchased from Ajinomoto, Co. (Tokyo, Japan), and concentrated glycerin was purchased from Kozakai Pharmaceutical Co. (Tokyo, Japan). Oleic acid of highest quality and cholesterol of reagent grade were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sorbitan monooleate and hydrogenated castor oil were purchased from Nikko Chemicals Co. (Tokyo, Japan). All other chemicals were of reagent grade and purchased from Wako Pure Chemical Industries.

Preparation of LE

The LE were prepared as follows: 720 mg of PC99 and cosurfactant (oleic acid, cholesterol, Tween 80, or HCO-60) were added to 4 g of soybean oil. This mixture was heated and stirred until completely clear. Thirty ml of water for injection and 884 mg of concentrated glycerin were added to the oil mixture. This mixture was pre-emulsified using a homogenizer (Model Physcotron NS-60, Niti-on Medical & Physical Instruments MFG. Co., Tokyo, Japan) at 8000 rpm for 10 min. Final emulsification was completed by passing the coarse emulsion through a high pressure homogenizer (Model French Miniature Pressure Cell, American Instrument Company, Urbana, USA). The pH of the coarse emul-

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sion was then adjusted to pH 6 with sodium hydroxide or hydrochloric acid aqueous solution. The volume of LE was adjusted to 40 ml with water for injection. During all processes, nitrogen gas was bubbled into the emulsion. The obtained LE were referred to as PC99LE-Ole, PC99LE-Cho, PC99LE-Tween80, and PC99LE-HCO60.

Measurements

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

Study of Relationship Between ζ Potential and pH

The above-described LE were diluted with aqueous potassium chloride solution (ionic strength, 0.0005), which had been adjusted to pH 4–9 using HCl or KOH. The mean electrophoretic mobility of each solution was measured at 25°C, and the ζ potential was calculated.

Storage Test at High Temperature

For the manufacturing process for injections, SUPS is generally requisite. Therefore, a stability test was performed with SUPS at 121°C for 20 min. LE containing various cosurfactants were filled in 1 ml ampoules. Samples were removed from SUPS over time, and their pH values and mean particle sizes were measured. In another study, LE-filled ampoules were immersed in an oil bath, and the temperature was maintained at 100 to 180°C, and the same kinds of measurements were carried out.

RESULTS AND DISCUSSION

Influence of Cosurfactants on ζ Potential and Mean Particle Size of LE

The values of ζ potentials and mean particle sizes of LE containing various cosurfactants are shown in Table I. To maintain a stable emulsion consisting of PC, its particle size had to kept under 250 nm, since we could not perform this study using LE with a particle size over 300 nm, because of fast separation. Based on these findings, we determined the concentrations of cosurfactants in Table I to obtain LE with a particle size of approximately 200 nm. The ζ potentials of PC99LE-Cho, PC99LE-HCO60, and PC99LE-Tween80 were −3 ~ −12 mV, while that of LE without cosurfactant (PC99LE) was −8 mV. That is, no effect of cosurfactants on the ζ potential was observed. In addition, the amount of cosurfactant had no effect on ζ potential. On the other hand, the ζ potential of PC99LE-Ole was −15 mV, and twice as large as that of PC99LE. The mean particle size of PC99LE-HCO60 was 130 nm, while that of the other emulsions was approximately 200 nm. It was considered that the particle size of PC99LE-HCO60 became small, since the surface tension of the emulsion was drastically decreased by the addition of HCO-60, which showed emulsification power. Lundberg (13) studied the particle sizes of LE containing PC, cholesterol and HCO-60. In this report, an LE with a particle size of 50 nm was prepared by adjustment of the cosurfactant ratio. In our study we prepared LE using PC and a single cosurfactant. However, if several combinations of cosurfactants are selected, small sized particles may be obtained. Furthermore, the conditions of emulsification, such as pressure and temperature may also be important factors for determining the particle size.

Table I. Parameters of LE Containing Cosurfactants

LE	Cosurfactant (mM)	ζ potential (mV)	Particle size (nm)	Vt^{max}/kT (kJ)
PC99LE		−8	204	2.6
PC99LE-Ole	3.5	−15	197	12.7
	7.0	−15	171	11.0
	17.5	−18	214	21.1
PC99LE-Cho	0.5	−12	182	6.8
	1.0	−12	212	8.0
PC99LE-HCO60	5.0	−9	126	2.2
	20.0	−3	90	−0.04
PC99LE-Tween80	0.8	−12	200	7.5
	4.0	−9	167	2.9

Using these findings, maximum total interaction energies (Vt^{max}) were calculated and are shown in Table I. In our previous study (9), the effect of Vt^{max} on flocculation was clarified. Vt^{max} was derived by DLVO theory and was the maximum of the total interaction energy of two identical spherical particles of radius a at separation H between their surfaces, $Vt(H)$, shown in Eq. 1:

$$Vt(H) = V_R + V_A(H), \tag{1}$$

where 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. V_R, is given by

$$V_R(H) = \frac{64\pi ankT\gamma^2}{\kappa^2}\exp(-\kappa H), \tag{2}$$

where n is the number density (in units of m^{-3}) of electrolyte in the bulk solution phase, k is the Boltzmann constant, T is the absolute temperature, and κ is the Debye-Hückel parameter. n, κ, and γ are defined as follows:

$$n = 1000 \cdot C \cdot N_A,\ \kappa^2 = \frac{2nz^2e^2}{\epsilon_r\epsilon_0 kT},\ \gamma = \frac{\exp(ze\zeta/2kT) - 1}{\exp(ze\zeta/2KT) + 1}, \tag{3}$$

where C is the concentration of electrolyte in the bulk solution phase, N_A the Avogadro number, z the ionic valence, e the electric unit charge, ϵ_r the relative permittivity of solution, ϵ_0 the permittivity of vacuum, and ζ the zeta potential. V_A can be calculated as:

$$V_A = -\frac{Aa}{12H,} \tag{4}$$

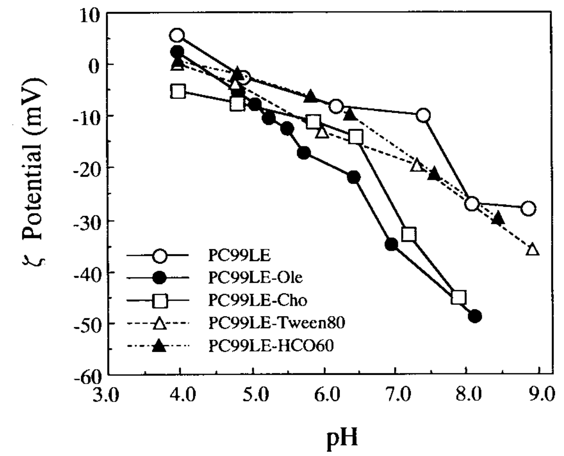

Fig. 1. ζ potentials of LE in 0.5 mM KCl aqueous solution (ionic strength, 0.0005) at various pHs. Adjustment to each pH was made with 0.5 mM KOH or 0.5 mM HCl. The mean electrophoretic mobility of each solution was measured at 25°C, and the ζ potential was calculated. Cosurfactant contents of PC99LE-Ole, PC99LE-Cho, PC99LE-Tween80, and PC99LE-HCO60 were 7.0 mM, 1.0 mM, 4.0 mM, and 5.0 mM, respectively.

where A is the Hamaker constant. A includes an energy level and is a factor which indicates the magnitude of the van der Waals interaction. We assumed no change in A by the addition of a small amount of cosurfactants. Two LE particles undergo flocculation when the $Vt^{\max}$ is lower than the thermal energy (kT).

Mervat *et al.* (14) and Handa *et al.* (6) reported liposome to be present in LE. This kind of study must be essentially performed by separating the lipid particle and the liposome. However, the ζ potential and A of the lipid particle could not be obtained, because it is very difficult to prepare LE without the liposome. Therefore in the present study, we assumed that the structures of the lecithin and cosurfactant on the lipid particle surface were similar to those on the liposome surface, and that the surface electric potentials on both the whole emulsion and the liposome had similar values. The $Vt^{\max}$ and ζ potentials of PC99LE-Cho, PC99LE-HCO60, and PC99LE-Tween80 were similar to those of PC99LE. The $Vt^{\max}$ of PC99LE-Ole was 11 ~ 21 kT, and seven times that of PC99LE. The ζ potentials of PC99LE-Tween80 and PC99LE-HCO60 did not change. Neither did their $Vt^{\max}$ change, since Tween 80 and HCO-60 are non-ionic substances. On the other hand, the ζ potentials of PC99LE-Cho and PC99LE-Ole were decreased and the $Vt^{\max}$ of those were increased, since the cholesterol and the oleic acid have a hydroxyl group and a carboxyl group, respectively. This finding suggested that PC99LE-Ole developed a large energy of attraction upon increasing the $Vt^{\max}$, and ceased being flocculated. Figure 1 shows pH-profiles of the ζ potential of LE. When the pH was below 6.5, the curves for PC99LE-Cho (1.0 mM of cholesterol), PC99LE-HCO60 (5.0 mM of HCO-60), and PC99LE-Tween80 (4.0 mM of Tween 80) coincided with that for PC99LE, but the absolute ζ potentials of PC99LE-Ole (17.5 mM of Oleic acid) were larger than those of the other LE. This difference between ζ potentials decreased with increasing pH. Washington *et al.* (5) have reported the effect of cosurfactants on flocculation. They indicated that LE emulsified using phosphatidylcholine and oleic acid decreases the ζ potential, but that the stability of this formula gradually decreases as storage time increases. Because the pH of LE gradually decreases, the ζ potential increases. These findings suggest that if the pH does not decrease, the oleic acid had the effect of increasing the potential energy of the electrostatic interaction and $Vt^{\max}$ at high pH. LE containing oleic acid therefore stabilizes with increasing pH.

Change in Mean Particle Size Induced by SUPS

Figure 2 shows the changes in mean particle sizes of LE induced by SUPS. The mean particle sizes of PC99LE-Cho, PC99LE-HCO60, and PC99LE-Tween80 after SUPS were more than twice those before SUPS. PC99LE-Tween80 displayed the largest change in the ratio of mean particle sizes. On the other hand, the ratio of change for PC99LE was 1.6. The findings suggested that these cosurfactants accelerated

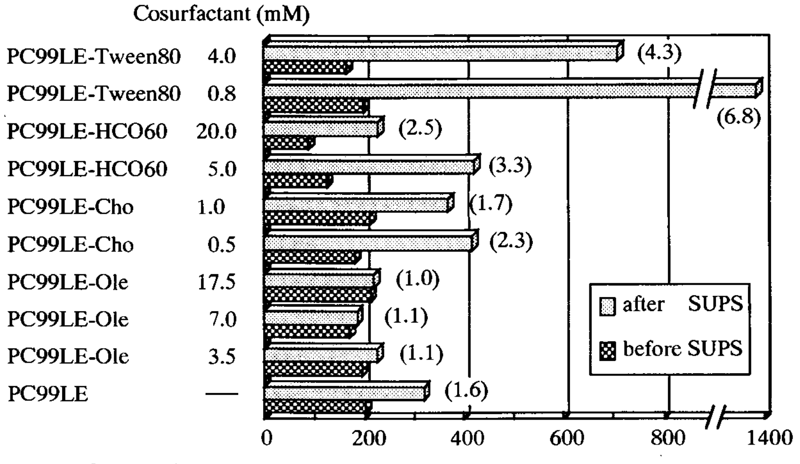

Fig. 2. Changes in mean particle size of LE containing various cosurfactants induced by SUPS. Values in ( ) show ratios of change (after SUPS/before SUPS) in mean particle size. LE containing various cosurfactants were filled in 1 ml ampoules, and were treated with SUPS at 121°C for 20 minutes.

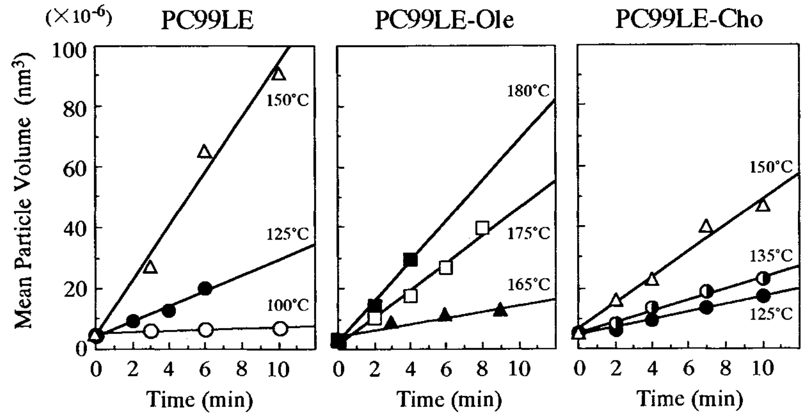

Fig. 3. Changes in mean particle volumes of LE with time of storage at various temperatures. Straight lines are regression curves determined using the method of least squares. LE containing various cosurfactants were filled in 1 ml ampoules, and were immersed in an oil bath at 100 to 180°C. Cosurfactant contents of PC99LE-Ole and PC99LE-Cho were 7.0 mM and 1.0 mM, respectively.

coalescence. However, the ratio of change for PC99LE-Ole was smaller than that for PC99LE, and coalescence of LE was not observed. Oleic acid suppressed the coalescence of LE. The repulsive interaction between the particles was thus increased by a large Vt^{max}, since the absolute ζ potential of LE was increased by the addition of oleic acid. These findings suggested that PC99LE-Ole did not undergo coalescence because flocculation became minimal, and that increase in particle size was suppressed.

Appearance Activation Energy of Coalescence (*E*) of LE

Lawrence and Mills (12) reported that the following equation can be used to predict the rate of increase in LE particle volume:

$$\frac{dv}{dt} = \frac{4kT}{3\eta}\phi\exp(-E/kT), \qquad (5)$$

where v is the mean particle volume, η the viscosity of the bulk solution, and ϕ the volume fraction of the bulk solution. Therefore, the appearance activation energy, E, which includes the energy for destruction of the interfacial membrane and the energy barrier for flocculation, Vt^{max}, can be obtained from the plot of the rate of increase in particle volume.

Oleic acid and cholesterol were used to stabilize the emulsion and to strengthen the liposome membrane, respectively. Therefore, we determined the thermodynamic parameters of PC99LE-Ole and PC99LE-Cho. Glass ampoules were filled with PC99LE-Ole (7.0 mM oleic acid), PC99LE-Cho (1.0 mM cholesterol), or PC99LE and placed in an oil bath at various temperatures. Figure 3 shows changes in LE particle volume. The curves thus obtained were linear against storage time. The rate of reaction increased with increasing temperature. The particle volumes of PC99LE and PC99LE-Cho increased to above 100°C, while those of PC99LE-Ole increased to 165°C and higher. PC99LE had the largest particle volume change, while PC99LE-Ole had the most stable particles.

These findings suggested that the increase in mean particle volume follows zero-order kinetics. When the number of lipid particles decreases, the rate constant, k, is proportionate to the approachable probability of the particles. It was considered that zero-order kinetics was shown as a result of decreased numbers of and the approachable probability of the particles. The dv/dt is plotted against $1000/T$ in Figure 4 to obtain the E from the slope of the lines. The E are shown in Table II. The E of PC99LE-Ole was 3.63×10^{-19} J (219 kJ/mole), and twice that of PC99LE; and the E of PC99LE-Cho was slightly smaller than that of PC99LE. The value of E is equal to the energy barrier to flocculation and coalescence. These findings suggest that oleic acid suppressed the reaction between lipid particles because of the large E induced.

Effect of Gosurfactants on Flocculation and Coalescence

The flocculation and coalescence of lipid particles can

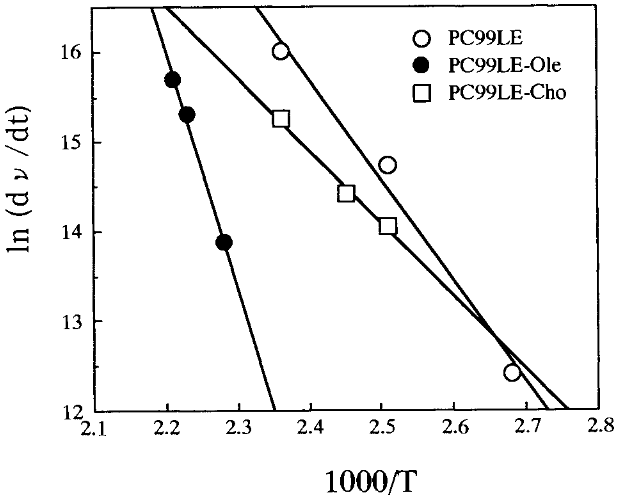

Fig. 4. Plots of $(dv\text{-}dt)/T$ in Eq. 5 for LE as a function of the reciprocal of absolute temperature. Straight lines are regression curves determined using the method of least squares. Cosurfactant contents of PC99LE-Ole and PC99LE-Cho were 7.0 mM and 1.0 mM, respectively.

Table II. Values of Vt^{max}, W, and E

LE	Cosurfactant (mM)	pH	$Vt^{max} \times 10^{19}$ (J)	$W \times 10^{19}$ (J)	$E \times 10^{19}$ (J)
PC99LE		6.2	0.141	1.36	1.50
PC99LE-Ole	7.0	5.8	0.598	3.03	3.63
PC99LE-Cho	1.0	6.1	0.435	0.63	1.06

be expressed using Vt^{max} and the energy barrier to coalescence, W, respectively. The total energy of flocculation and coalescence can be calculated as

$$E = Vt^{max} + W. \tag{6}$$

The values of Vt^{max}, W, and E per molecule are shown in Table II. The values of W of PC99LE-Ole and PC99LE-Cho were respectively twice and half that of PC99LE. These findings indicate that the Vt^{max} of PC99LE was smaller than those of PC99LE-Ole and PC99LE-Cho, while the W of PC99LE was not small. PC99LE thus readily underwent flocculation, but underwent little coalescence. The Vt^{max} of PC99LE-Cho was relatively large, while its W was very small. PC99LE-Cho thus underwent little flocculation but was easily coalesced if two particles underwent flocculation. On the other hand, the Vt^{max} and W of PC99LE-Ole were large. PC99LE-Ole underwent little flocculation and little coalescence. Oleic acid appeared to suppress the flocculation and coalescence of lipid particles, while cholesterol appeared to suppress only flocculation, suggesting that the cholesterol or the oleic acid formed the condensed lecithin membrane, and that the oleic acid decreased the surface negative charge by its dissociation.

The W of LEs was larger than their Vt^{max}, and was similar in value to E. This finding indicated that even if LE had undergone flocculation, it underwent little coalescence. Therefore, a flocculated parenteral lipid emulsion will be redispersed by shaking. It was reported that cholesterol appeared to induce coalescence of liposomes (15). It was found that this effect was not due to suppression of coalescence, but to flocculation of the liposome particles.

CONCLUSION

The Vt^{max} of LE was increased three times by the addition of cholesterol, but W was reduced to one-half its former value. It thus appeared that cholesterol prevented flocculation but did not prevent coalescence. On the other hand, the Vt^{max} and W of LE were increased four times and twice, respectively, by the addition of oleic acid. It thus appeared that oleic acid prevented flocculation and coalescence. Two different cosurfactants had different effects on the stabilization of the lipid emulsion. Flocculation and coalescence should be determined separately using the maximum total energy, Vt^{max}, and the energy barrier to coalescence, W. These parameters were considered useful as indices of the stability of LE.

ABBREVIATIONS

LE	lipid emulsion
PC	egg yolk phosphatidycholine
SUPS	steam under pressure sterilization
Vt^{max}	maximum total interaction energy
E	appearance activation energy for coalescence
W	energy barrier for coalescence
PC99	99% purified phosphatidylcholine
Tween 80	sorbitan monooleate
HCO-60	hydrogenated castor oil
PC99LE	LE without cosurfactants
PC99LE-Ole	LE containing an oleic acid
PC99LE-Cho	LE containing a cholesterol
PC99LE-Tween80	LE containing Tween 80
PC99LE-HCO 60	LE containing HCO-60

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