O/W-Emulsion Stabilized by Hydrophobized Polysaccharide

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Colloidal stability of oil-in-water (o/w)-emulsion of α-linolenic acid (ALA) with or without trioctanoylglycerol (TriC$_8$) was investigated in the presence of a hydrophobized polysaccharide, cholesteryl-bearing pullulan (CHP). The addition of ALA made the CHP/TriC$_8$-emulsion more colloidally stable. A smaller and more colloidally stable o/w-emulsion was formed with an ALA/TriC$_8$/CHP mixture. Electrophoretic light scattering measurements showed that the surface potential of the CHP/ALA/TriC$_8$-emulsion was approximately zero. Fluorescence depolarization measurement using fluorescein isothiocyanate (FITC)-labeled CHP revealed that CHP adsorbs onto the surface of oil droplets and stabilizes the emulsion by a steric repulsion mechanism.

An emulsion is defined as a liquid–liquid dispersion system in which one of two liquids is dispersed in another immisible liquid to give microsize or nanosize droplets. When the bulk phase is water, the emulsion formed is called oil-in-water (o/w) emulsion. The emulsion is widely used in industries such as cosmetics, foods, medicines, and agricultural chemicals. For preparation of o/w-emulsion, an emulsifier is usually added into the aqueous medium as a protective reagent to increase colloidal stability of the emulsion produced. The most general emulsifier is a surfactant. Water-soluble polymers also have been extensively employed in practical applications.

In general, emulsions gradually disrupt with time because of thermodynamic instability. Important factors to maintain the stability of emulsions are (i) interfacial tension, (ii) electrostatic repulsion, and (iii) steric repulsion. Surfactant drastically lowers the interfacial tension upon its adsorption to liquid–liquid interface and facilitates formation and stabilization of the emulsion. In addition, by using an ionic surfactant, the emulsion is stabilized by the electrostatic repulsion mechanism. With polymers, the ability to decrease the interfacial tension is much lower compared with that of surfactants. However, each nonionic polymer provides a layer on the particle surface upon adsorption, and, thereby, the emulsions are stabilized by steric repulsion mechanism. Stabilization of o/w-emulsion by water-soluble polymers has been extensively investigated from theoretical and experimental standpoints. They are usually water soluble polymers such as gums,[1—3] proteins,[4] or Pluronics.[5] However, few systematic studies have been reported which include graft co-polymers as the emulsifier.[6]

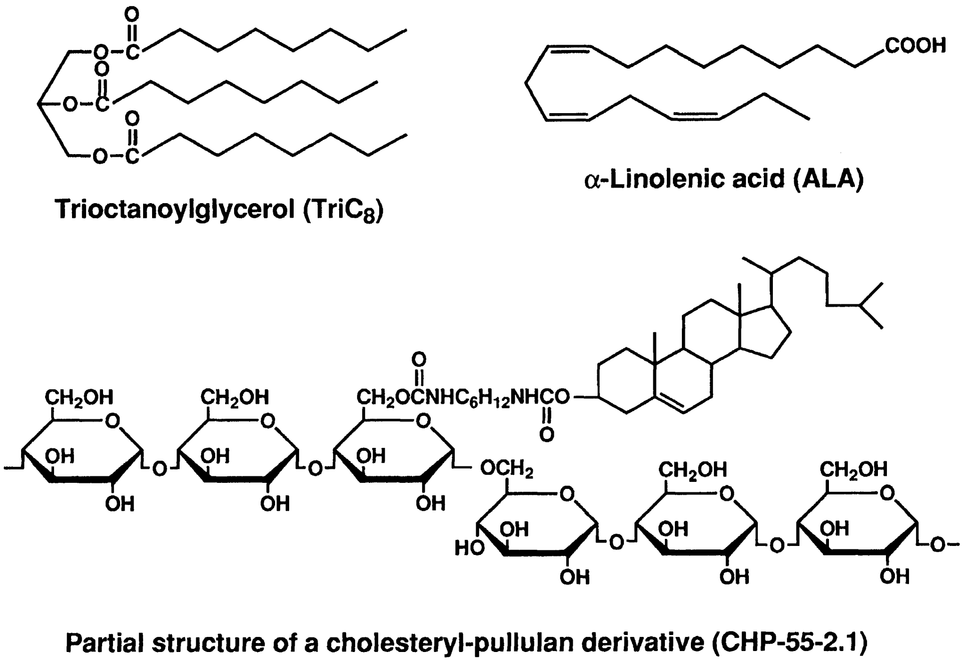
Since 1982, we have developed hydrophobized polysaccharides and applied them to biotechnology and medicine. They are naturally occurring polysaccharides such as pullulan, dextran, mannan, amylose, and amylopectin, those are partly hydrophobized with palmitoyl or cholesteryl moieties. Among these hydrophobized polysaccharides, cholesteryl-bearing pullulan (CHP) was found to effectively coat liposomal surface,[7] lecithin emulsion,[8,9] lipid monolayer,[10,11] and black lipid membrane.[12] Moreover, a mixture of CHP and trioctanoylglycerol (TriC$_8$) gave an almost monodispersive emulsion in water upon ultrasonic irradiation.[13]

In this work, the stabilization mechanism of an o/w-emulsion composed of α-linolenic acid (ALA), TriC$_8$, and CHP (Fig. 1) was investigated by dynamic light scattering, electrophoretic light scattering, interfacial tension, and fluorescence depolarization. Here, ALA, which is used as an oil phase of the emulsion, has attracted much attention as a safe anticancer agent because of its high selective cytotoxicity against cancer cells.[14—17] Therefore, the physicochemical property of the CHP-stabilized o/w-emulsion is very important to develop a new carriers for lipophilic drugs such as ALA.

Materials and Methods

ALA was purchased from Sigma Chemical Co., St. Louis, MO, while TriC$_8$ was supplied from Nippon Oil & Fats, Co., Ltd. Purities of ALA and TriC$_8$ were 98% and 99.6%, respectively. In this study water used was processed through a Milli-Q system (Millipore Corp., Bedford, MA) after careful distillation. CHP-55-2.1 was exactly the same as that employed in previous works;[18,19] pullulan ($M_W = 55000$) as substituted by 2.1 cholesteryl moieties per 100 glucose units (Fig. 1). CHP-55-1.6 and pullulan-55 labeled with fluorescein isothiocyanate (FITC) were prepared according to the method previously described.[20] The substitution degree of FITC moiety per 100 glucose units was 0.49 for CHP and 0.55 for pullulan; FITC (0.49)-CHP-55-2.1 and FITC (0.55)-P-55.

Preparation of Emulsions. The emulsions employed in this work were prepared as follows. Briefly, CHP (ca. 1.0 mg ml^{-1}) was thoroughly suspended and swelled in a phosphate-buffered saline (pH = 7.4, 0.80% NaCl, 0.02% KCl, 1.15% Na$_2$HPO$_4$, and 0.02% KH$_2$PO$_4$) under stirring for 24 h at 45 °C. The resulting suspension was sonicated using a probe type sonifier (Tomy UD-201, Tokyo)



Trioctanoylglycerol ($TriC_8$)

α-Linolenic acid (ALA)

Partial structure of a cholesteryl-pullulan derivative (CHP-55-2.1)

Fig. 1. Structures of chemicals employed in this work.

for 5 min at 40 W and room temperature. An ethanolic solution of ALA (26.7 $mg\,ml^{-1}$) and/or $TriC_8$ (26.7 $mg\,ml^{-1}$) was added into the aqueous CHP suspension, and the resulting mixed suspension was sonicated again under N_2-atmosphere for 10 min at 40 W and 0 °C. ALA, $TriC_8$, or an ALA/$TriC_8$ mixture (1 : 9—9 : 1 by wt) was used as the oil phase. A FITC/CHP or FITC/pullulan-emulsion was also prepared according to the same procedure. All the sample emulsions thus prepared were stored in the dark at 4.0 °C.

Particle Size. The particle size of the emulsion was measured by dynamic light scattering (DLS) on a DLS-700 (Otsuka Electronics Co., Osaka) equipped with a bath (RTE-110, Neslab) thermoregulated at 25.0 °C. Measurements were carried out after appropriate dilution of the sample emulsion with PBS. The autocorrelation function was analyzed by the cumulant method. Colloidal stability of the emulsion was evaluated from the particle size change of the sample when stored at 4.0 °C.

For all the particle size measurements, the sample emulsion was quickly warmed up to 25.0 °C on a thermoregulated bath just before the measurement. If needed, the sample was further diluted by a given amount of PBS buffer solution prewarmed at 25.0 °C in advance. All the size measurements were carried out at 25.0 °C. It was confirmed in advance that this heating process of the sample emulsion does not significantly affect the particle size change under the conditions employed.

Interfacial Tension. Tension at the oil/water interface was measured at 24.0±0.5 °C using a Wilhelmy-type automated surface tensiometer (CBVP-A3, Kyowa Interface Science Co., Ltd., Tokyo) with a platinum plate. $TriC_8$ of a $TriC_8$/ALA (9 : 1 by wt) mixture was used as the oil phase. Milli-Q water, PBS, or an aqueous CHP suspension (1.0 $mg\,ml^{-1}$) was employed as the water phase.

Zeta Potential. For zeta potential measurements, the ALA/$TriC_8$-emulsion (ALA : $TriC_8$ = 7 : 3 by wt and total oil 1.51 $mg\,ml^{-1}$), the CHP/ALA/$TriC_8$-emulsion (CHP : ALA : $TriC_8$ = 5 : 7 : 3 by wt and CHP 0.75 mg + total oil 1.51 $mg\,ml^{-1}$) and the CHP/$TriC_8$-emulsion (CHP : $TriC_8$ = 1 : 2 by wt and CHP 0.75 mg + $TriC_8$ 1.51 $mg\,ml^{-1}$) were diluted 10—50 times with either PBS or 10 mM NaCl. The resulting sample was placed into a measuring cell by direct filtration through a membrane filter (pore size = 1.2 μm, Gelman Science, Michigan). Zeta potential was measured at 25.0 °C by electrophoretic light scattering (ELS) method on an ELS-800 (Otsuka Electronics Co., Osaka) according to a well-authorized method.[21]

Bulk pH of Emulsion. All the samples such as the CHP/ALA/$TriC_8$-emulsion (CHP : ALA : $TriC_8$ = 5 : 7 : 3 by wt, CHP 0.75 mg + total oil 1.51 $mg\,ml^{-1}$), the CHP/$TriC_8$-emulsion (CHP : $TriC_8$ = 1 : 2 by wt, CHP 0.75 mg + $TriC_8$ 1.51 $mg\,ml^{-1}$), and the ALA/$TriC_8$-emulsion (ALA : $TriC_8$ = 7 : 3 by wt, [total oil] = 1.51 $mg\,ml^{-1}$) were diluted 7 times with PBS. The bulk pH of the emulsion was adjusted to be 4—9 using an aqueous 0.1 M HCl or 0.1 M NaOH solution (1 M = 1 $mol\,dm^{-3}$). After incubation for 1 h at 25.0 °C, the turbidity at 360 nm was measured on a Hitachi 220A spectrophotometer (Hitachi, Tokyo), while the particle size of the emulsion was by DLS.

Coating of Oil Droplets with CHP. To evaluate the interaction between oil droplets and CHP, the steady-state fluorescence depolarization was studied for FITC-CHP or FITC-pullulan as mixed with various oil components such as ALA, $TriC_8$, and ALA/$TriC_8$ (7 : 3 by wt). The weight ratio of FITC-polysaccharide to the oil was adjusted to 0.1. The emulsions prepared with FITC-CHP or FITC-pullulan were diluted with PBS to an appropriate FITC concentration for the fluorescence depolarization measurement. The fluorescence depolarization measurements were carried out on a Union Giken FS-501S fluorescence depolarization spectrophotometer combined to a Komatsu-Yamato Coolnics Model CTR-120 at 25.0 °C. The probe FITC was exited at 440 nm, and the fluorescence was collected using a sharp cut-off filter Y-46 (Hoya Glass Works, Tokyo) to completely eliminate the fluorescence at wavelength below 440 nm. The steady state fluorescence depolarization value, p-value, can be obtained from Eq. 1:

$$p = (I_{VV} - C_f I_{VH})/(I_{VV} + C_f I_{VH}), \qquad (1)$$

where I is the fluorescence intensity, and subscripts V and H indicate the vertical and horizontal orientations of excitation (first) and analyzer (second) polarizers, respectively. $C_f(=I_{VH}/I_{HH})$ is a grating correction factor.

Results and Discussion

Stability of o/w-Emulsion Stabilized by CHP. First, the particle size of the o/w-emulsion stabilized with CHP was investigated by DLS. Figure 2 shows changes of particle size for the CHP/ALA/TriC$_8$-emulsion just after preparation under ultrasonication. The particle size of the emulsion depended on the content of both ALA and TriC$_8$ as the oil phase. Smaller particles were obtained by coexistence of ALA and TriC$_8$. Ease of emulsification was closely related to the particle size of the emulsion. Therefore, formation of smaller particles was accompanied by easier emulsification. In general, less hydrophobic oils are considered to be hardly emulsified. Therefore, the larger particle size of the CHP/ALA-emulsion compared with the CHP/TriC$_8$ and the CHP/ALA/TriC$_8$ emulsions would be ascribed to its water solubility of ALA being higher than that of TriC$_8$ (vide supra). The particle size of the emulsion gradually increased during the storage at 4.0 °C (Fig. 3). In the case of the CHP/ALA-emulsion, it was impossible to measure the accurate particle size of the emulsion at 5 d after emulsification because of significant precipitation. Figure 4 shows an influence of the oil component on the particle size change of the emulsion (Δ particle size), which is a difference between the particle size of emulsion just after preparation and that left for 5 d or 1 month. Colloidal stability of the emulsion significantly increased by an appropriate blend between ALA and TriC$_8$. Especially, when the ALA/TriC$_8$ ratio was 7 : 3 (by wt), the emulsion was most colloidally stable. The CHP/ALA-emulsion was instable compared with the CHP/TriC$_8$- or the CHP/ALA/TriC$_8$-emulsion. Avranas et al. investigated the effect of sodium dodecyl sulfate[22] and benzylhexadecylethylammonium chloride[23] on the colloidal stability of o/w-emulsions using octane, isooctane, or methylcyclohexane and a nonionic surfactant (Tween 80). They described that the higher the solubility of the oil in water was, the lower the stability of the emulsion was.[22,23] Moreover, Rehfeld has reported that the colloidal stability of emulsions is very low when prepared with oils those have a large spreading coefficient (S_0). This parameter (S_0) is obtained from Eq. 2.[24]

$$S_0 = \sigma_w - (\sigma_{ow} + \sigma_0), \tag{2}$$

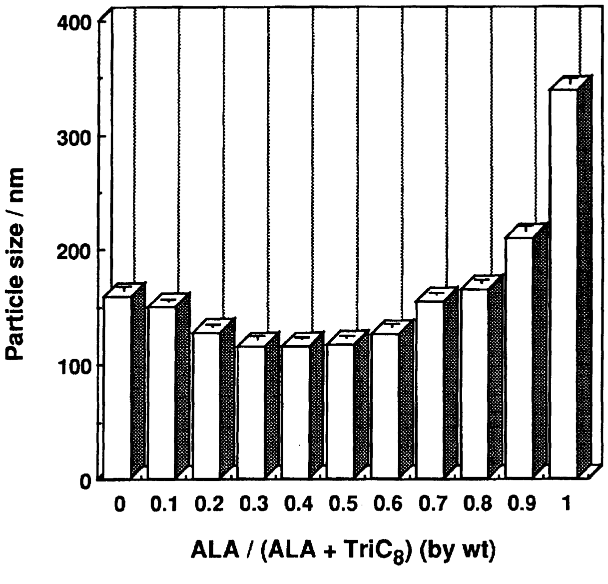


Fig. 2. Effect of lipid composition on particle size just after emulsification of CHP/ALA/TriC$_8$-emulsion at 25.0 °C in PBS; (CHP)/(ALA+TriC$_8$)=0.5 by wt.

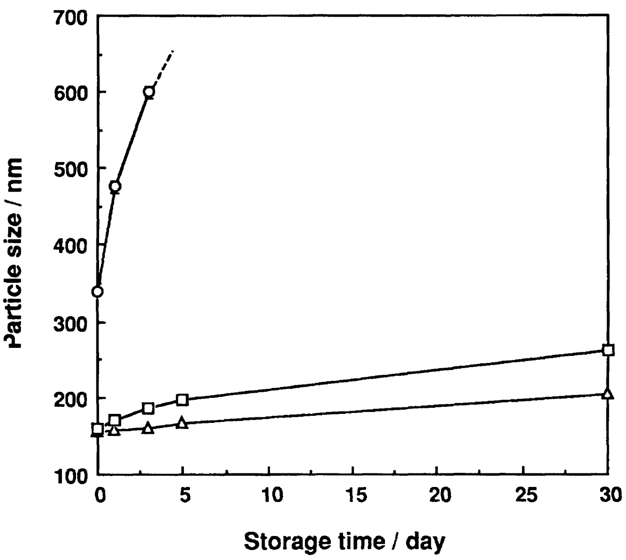


Fig. 3. Particle size change of CHP/ALA (5 : 10 by wt)-emulsion (○), CHP/TriC$_8$ (5 : 10 by wt)-emulsion (□), and CHP/ALA/TriC$_8$ (5 : 7 : 3 by wt)-emulsion (△) as a function of storage time at 4.0 °C. Size was measured at 25.0 °C.

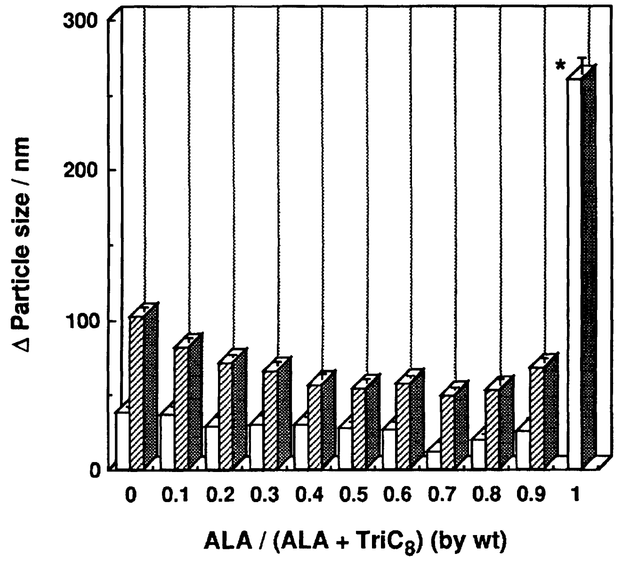


Fig. 4. Effect of lipid composition of oil phase on colloidal stability of CHP/ALA/TriC$_8$-emulsion in PBS. Samples were stored at 4.0 °C; CHP/(ALA+TriC$_8$)=0.5 by wt. Delta particle size means the difference in the particle size between just after the emulsification and 5 d later (□) or 1 month later (■). *Difference in the particle size between just after emulsification and after 3 d.

where σ_w is the surface tension of water, σ_{ow} is the interfacial tension at the oil/water interface, and σ_0 is the surface tension of the oil. An oil with a large S_0 shows high water solubility.[24] The oil with higher water-solubility may tend to easily penetrate into the adsorbed surfactant layer[25] and into the adjacent interfacial fluid, leading to rapid coalescence of the oil droplets.[24] As a result, S_0 of ALA was larger than that of TriC$_8$; S_0=22.9±1.4 dyne cm^{-1} for ALA, while S_0=17.9±0.8 dyne cm^{-1} for TriC$_8$. Consequently, the lower colloidal stability of the CHP/ALA-emulsion compared with the CHP/TriC$_8$-emulsion could be attributed to the higher water solubility of ALA than that of TriC$_8$.

With the CHP/TriC$_8$-emulsion and the most stable CHP/ALA/TriC$_8$-emulsion (ALA/TriC$_8$=7 : 3 by wt), we examined the effect of the CHP concentration on both the particle size and the colloidal stability. Figure 5 shows the effect of the amount of CHP on the particle just after the emulsification. For the CHP/TriC$_8$-emulsion the particle size decreased with an increase in the amount of CHP and was almost unchanged above the CHP/oil ratio 0.1. Of course, emulsions were not formed without CHP. For the CHP/ALA/TriC$_8$-emulsion, on the other hand, the particle size was almost independent of the CHP concentration. Moreover, ALA/TriC$_8$-emulsion was formed even without CHP; the size was approximately 150 nm. Figure 6 shows a relation between the particle size change and the CHP/oil weight ratio. The CHP/TriC$_8$-emulsion showed high colloidal stability, and the stability did not depend on the CHP/oil ratio above 0.02. However, the stability drastically decreased below 0.01 of the CHP/oil ratio. The CHP/ALA/TriC$_8$-emulsion became more stable as the CHP/oil ratio increased. The emulsion became stable enough by the addition of a very small amount of CHP. These results suggest that coexistence of ALA and TriC$_8$ gives smaller and more stable-emulsions. In addition, the composition of the oil phase also affects the interaction between the oil droplets and the polymer stabilizer.

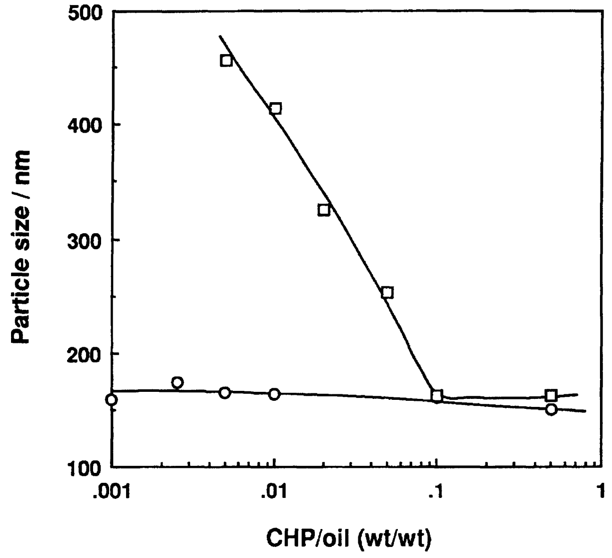


Fig. 5. Effect of CHP/total oil-ratio on particle size just after emulsification with CHP/TriC$_8$ (5 : 10)-emulsion (□) and CHP/ALA/TriC$_8$ (5 : 7 : 3 by wt)-emulsion (○) at 25.0 °C in PBS.

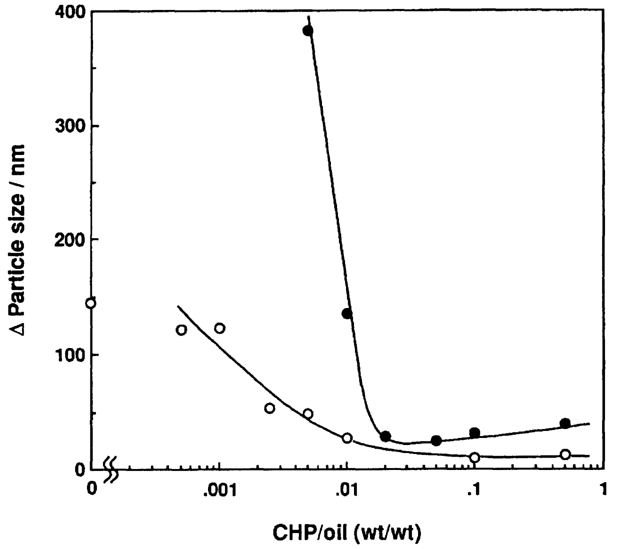


Fig. 6. Effect of CHP/lipid-ratio on colloidal stability with CHP/TriC$_8$ (5 : 10 by wt)-emulsion (●) and CHP/ALA/TriC$_8$ (5 : 7 : 3 by wt)-emulsion (○) in PBS at 4.0 °C. The particle size change was measured at 25.0 °C just after the emulsification and after 7 d.

Interfacial Tension. In general, the surfactants reduce surface and interfacial tension. Therefore, addition of a surfactant facilitates the emulsification and stabilizes the emulsion by lowering the interfacial tension. In addition to cholesterols,[26] lipids,[3,26] and monoglycerides,[3,27—29] free fatty acids such as oleic acid also are known to lower the interfacial tension of *n*-dodecane/water system.[26] In our present system, CHP is regarded as a polymer surfactant. Therefore, to determine whether ALA and/or CHP can behave as the surfactant, the interfacial tension of the TriC$_8$/PBS system was measured in the presence or absence of ALA and CHP (Table 1).

A significant decrease in the interfacial tension (approximately 10 dyne cm^{-1}) was observed at the TriC$_8$/PBS interface when 10.0 wt% ALA was added to the TriC$_8$ phase. This strongly suggests that ALA locates at

Table 1. Effect of ALA and/or CHP on Interfacial Tension of TriC$_8$/Milli-Q Water and TriC$_8$/PBS Systems at 24.0±0.5 °C

System	Interfacial tension
	dyne cm^{-1}
TriC$_8$/PBS	18.3±0.1
TriC$_8$/Milli-Q water	24.9±0.2
0.1 wt% CHP dissolved in PBS phase	17.8±0.1
10.0 wt% ALA mixed to TriC$_8$/Milli-Q water	16.0±0.2
10.0 wt% ALA mixed to TriC$_8$/PBS	8.2±0.4
10.0 wt% ALA mixed to TriC$_8$/PBS +0.1 wt% CHP dissolved in PBS	7.7±0.3

the $TriC_8$/PBS interface and acts as the surface active agent. This was seen also when Milli-Q water was employed for PBS. Therefore, the formation of smaller and more stable emulsion with ALA is ascribed to the role of ALA as the surfactant. With the $TriC_8$/PBS and the (ALA+$TriC_8$)/PBS systems, on the other hand, only a small change in the interfacial tension was observed even if CHP was added. It is already known that CHP forms a colloidally stable self-aggregate in water.[18,19] Though the CHP self-aggregates rather rapidly adsorb at the oil/water interface,[30] the further strong interaction accompanied by penetration of the cholesteryl moiety into the oil phase is extremely slow and takes a longer time.[10,11] Therefore, the less interfacial tension change with the CHP addition (within a short time and without vigorous agitation) must be ascribed to the rather slow penetration of the cholesteryl moiety of CHP into the $TriC_8$ phase.

Surface Potential. Both steric and electrical repulsions are important to stabilize colloidal dispersions. Therefore, zeta potential of the emulsion as stabilized with CHP was measured by ELS[21] (Table 2). With the $TriC_8$-emulsion, the addition of ALA made the surface potential negative. This would be due to the acid dissociation of ALA that localizes on the oil droplet surface. On the other hand, the surface charge of the CHP/ALA/$TriC_8$-emulsion droplet was almost neutral within experimental error. This strongly suggests that CHP adsorbs and coats the ALA/$TriC_8$-droplet surface. Carlsson et al. have already reported that coating of negatively charged lipid microspheres with CHP reduces its zeta potential to approximately zero.[8]

Effect of bulk pH on the stabilization of emulsion was investigated to understand the effect of ALA. As shown in Fig. 7, the particle size and the turbidity of the ALA/$TriC_8$-emulsion significantly increased below pH 6.0. This can be ascribed to a decrease in the negative charge of the emulsion surface. Therefore, the significant increase of the particle size and the turbidity of the emulsion below pH 6.0 would be due to the coalescence among colloid particles upon the decrease in the electrostatic repulsion. On the other hand, the existence of CHP did not much affect the particle size and the turbidity of the ALA/$TriC_8$-emulsions. This also indicates that CHP effectively coats the surface of the oil droplet. Taking all the results into account, we can not explain the stabilization of the emulsion with CHP by the electrostatic effect.

Interaction between Oil Droplets and CHP. There are two mechanisms for the stabilization of colloidal particles by polymers. One is the steric repulsion between polymer layers absorbed on the colloidal particle surface. Another is the depletion effect, which involves the effect of nonadsorbable polymers.[31] When colloidal particles approach closer to each other, a region into which the soluble polymers can not enter is formed between the particles. Consequently, the aggregation between the particles is facilitated. However, in the process of depletion restabilization, the aggregation of colloidal particles is depressed by the penetration of miscible polymers into the region provided between the particles. In order to propose a plausible mechanism for the emulsion stabilization with CHP, the interaction between the oil droplet and CHP was evaluated from the mobility change of CHP. For this purpose, the emulsions were prepared by using fluorescein-labeled CHP (FITC-CHP), and the p-value of FITC moiety was determined by the fluorescence depolarization method. Sunamoto and his co-workers have already investigated the restriction of molecular motion of the hydrophobized polysaccharides on liposomal surface by the

Table 2. ζ-Potential of o/w-Emulsions

Emulsion	Water phase	ζ-Potential (mV)
CHP/ALA/$TriC_8$ (5/7/3 by wt)	PBS	+2.69 (±3.09)
	10 mM NaCl	−0.86 (±1.86)
CHP/$TriC_8$ (1/2 by wt)	PBS	+5.37 (±1.27)
	10 mM NaCl	+1.93 (±0.46)
ALA/$TriC_8$ (7/3 by wt)	PBS	−23.65 (±1.14)
	10 mM NaCl	−53.03 (±0.42)

PBS; I=0.194 and pH 7.4.

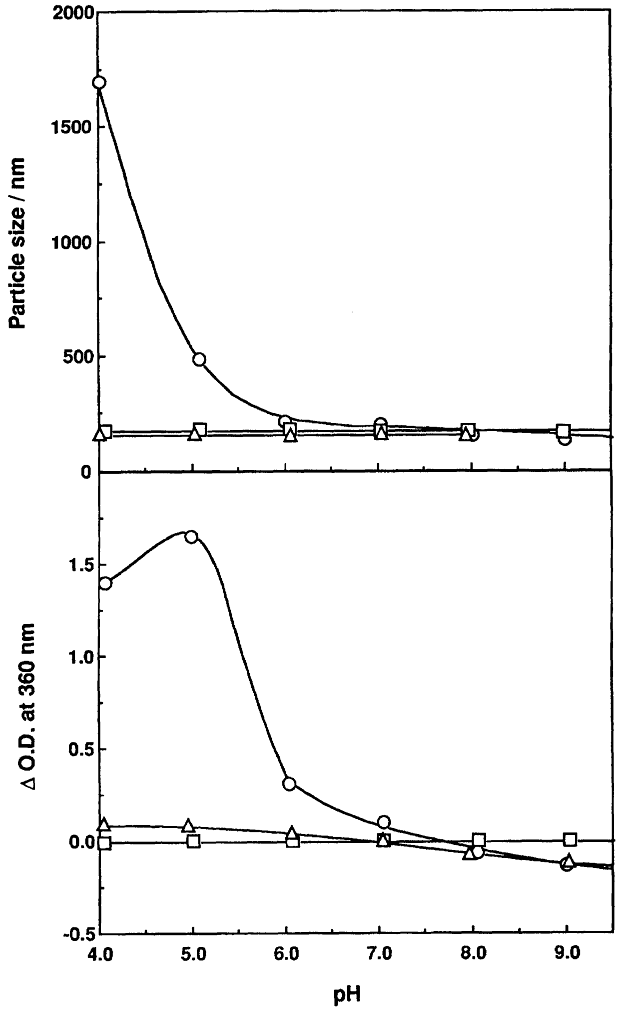


Fig. 7. Change of particle size and turbidity as a function of bulk; for ALA/$TriC_8$-emulsion (○), CHP/$TriC_8$-emulsion (□), and CHP/ALA/$TriC_8$-emulsion (△). Optical density change (Δ O.D.) at 360 nm was measured pH at 25.0 °C just after emulsification and after 1 h.

Table 3. Particle Size, Colloidal Stability (Expressed as Δ Particle Size), and *p*-Values of FITC-CHP or -Pullulan Various Emulsions (25.0 °C)

Sample	Particle size nm	Δ Particle size[a)] nm	*p*-Value
FITC-CHP	—	—	0.10±0.00
FITC-CHP/ALA/TriC$_8$	154±10	29±1	0.18±0.00
FITC-CHP/TriC$_8$	180±9	52±1	0.24±0.01
FITC-Pullulan	—	—	0.10±0.00
FITC-Pullulan/ALA/TriC$_8$	150±6	120±5	0.19±0.01
FITC-Pullulan/TriC$_8$	N.D.[b)]	N.D.[b)]	0.11±0.00

a) Δ Particle size is the difference in the size between just after preparation and 7 d later. b) N.D.; not determined.

method using FITC-labeled polysaccharide derivatives.[7,32)] Table 3 shows *p*-values of the FITC moiety conjugated to CHP or pullulan when mixed with different oil phases. The particle size and the colloidal stability of the emulsions were also shown in Table 3. *p*-Values of FITC moiety of the FITC-CHP-emulsion were higher than that of free FITC-CHP in an aqueous solution. This means that the molecular motion of CHP is restricted upon the adsorption on the oil droplets surface. Even with FITC-pullulan, an increase in the *p*-value was certainly observed for the FITC-pullulan/ALA/TriC$_8$-emulsion. On the other hand, when only TriC$_8$ was used as the oil phase, the *p*-value change was extremely small even if FITC-pullulan was added, and the formed emulsion was not stable. This means that pullulan itself hardly interacts with TriC$_8$.

Judging from all the results obtained, we suggest that the o/w-emulsions prepared by using CHP are stabilized by steric repulsion mechanism between polysaccharide layers adsorbed on the oil droplets surface. The interaction between the oil droplets and CHP is, however, affected by the composition of the oil phase. With the TriC$_8$-emulsion, the polysaccharide skelton of CHP hardly interacts with the oil droplets, and the cholesteryl moiety of CHP seems to play an important role for the absorption of CHP onto the oil droplets surface. With the ALA/TriC$_8$-emulsion, both the cholesteryl moiety and the pullulan skelton of CHP contribute to the stabilization of the emulsion (Table 3).

Conclusions

The ternary CHP/ALA/TriC$_8$-emulsion provided smaller particle size and higher colloidal stability than binary CHP/TriC$_8$-emulsion or the CHP/ALA-emulsion. The lower colloidal stability of the CHP/ALA-emulsion was due to the higher water solubility of ALA. The interfacial tension measurement revealed that ALA itself stabilized the oil droplets as the surface active agent in the CHP/ALA/TriC$_8$-emulsion system. The stabilization of the emulsions prepared with CHP was attributed to steric repulsion of polysaccharide layers that were formed at the oil droplets surface upon the coating.

We have preliminarily reported that the CHP/ALA/TriC$_8$-emulsion shows high antitumor activity to MM46 solid tumor-bearing mice by systemic administration.[33)] Therefore, the o/w-emulsion stabilized by the hydrophobized polysaccharides is promising as an improved delivery system for lipophilic drugs.

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