Effect of Polymer Additives on the Stability of O/W Emulsions

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Different types of polymers were added to ethyl oleate-in-water emulsions and the stability of the emulsions was followed by a turbidimetric technique. Addition of neutral polymers such as pullulan and dextran at 1% (w/v) destabilized the emulsions. The dependence of the stability of the emulsions on pullulan concentration was examined. When the concentrations were lower than 0.5% (w/v) and higher than 8.5%, the stability was almost the same as that at 0%. At the concentrations of 1 to 7%, the emulsions were destabilized. These phenomena are discussed based on a model in which the depletion effect was incorporated into a model derived from a balance of forces working on a coalescing droplet.

Keywords: O/W emulsions, depletion effect, neutral polymer

The stability of emulsions is an important and complicated problem in food manufacturing. In most cases, food emulsions include both small and large molecules as well as emulsifier. Macromolecules such as xanthan gum are often added to emulsions as stabilizers. The addition or presence of macromolecules in oil-in-water emulsions affects the stability of emulsions, e.g., adsorption of the macromolecules at the interface (Courthaudon *et al.*, 1991), depletion effect (Feigin & Napper, 1980; Fleer & Scheutjens, 1987), etc. These effects are usually examined at relatively low concentrations of additives.

Encapsulation of lipid droplets in a powdery matrix of an entrapping agent is achieved by emulsification of the lipid with a dense solution of the entrapping agent and by subsequent rapid dehydration of the O/W emulsions (Imagi *et al.*, 1990). The stability of the emulsions during dehydration is a crucial factor for successful encapsulation. The progress of dehydration brings about an increase in the concentration of the entrapping agent in the continuous phase of O/W emulsions.

Previously, we proposed a model to estimate the stability of emulsions in a cream layer (Adachi *et al.*, 1992) derived from a balance of forces operating on a particle based on the DLVO theory and Stokes' law. The model predicted that increases in viscosity of the continuous phase stabilized emulsions. Because the addition of a polymer increases viscosity, it is expected that such an addition would stabilize the emulsions. However, no experimental verification of this point has yet been reported. The stability of emulsions over a wide range of polymer concentrations has not been examined experimentally, although some theoretical studies have been reported (Asakura & Oosawa, 1954; Fleer & Scheutjens, 1987).

In this study, we investigated the effects of addition of polymer at various concentrations on the stability of emulsions and propose here a model to explain the effect of addition of a neutral polymer.

Materials and Methods

Materials Ethyl oleate (purity>98%) and Tween 20 were purchased from Wako Pure Chemicals, Osaka. Pullulans with different molecular weights (number-averaged molecular weights $M_n = 6.5 \times 10^4$, 9.2×10^4 and 2×10^5 (PI-20)) were gifts from Hayashibara Co., Okayama. Dextran T-10, T-40, T-70 and T-500, the $M_{\rm n}$ s of which were 5.1×10³, 2.65× 10^4 , 3.77×10^4 and ca. 4×10^5 , respectively, were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. The M_n values were determined by the manufacturers. DEAE- and SP-Dextrans were also obtained from Pharmacia Fine Chemicals. Maltodextrin with dextrose equivalent (DE) of 2-5 was obtained from Matsutani Chemicals, Osaka. Gum arabic, sodium caseinate and egg albumin were purchased from Nacalai Tesque, Kyoto. These polymers were used as received. Other chemicals were purchased from Wako Pure Chemicals and were of analytical grade.

Emulsion preparation and examination of stability Preparation of emulsions and analyses of their stability were carried out under two different conditions. In the first case, which will be referred to as condition A, 2.5 g of ethyl oleate and 25 ml of 0.02% (w/v) Tween 20 dissolved in water were placed in a 50-ml beaker and homogenized for 1 min with a Polytron PCU-2 (Kinematica, Lucerne, Switzerland) at a power setting of 7 to prepare O/W emulsions. The median diameter of the emulsions, which was evaluated using a Shimadzu SA-CP3L centrifugal particle analyzer (Kyoto), was about 9 μ m. The diameter was slightly different in each preparation. One ml aliquots of the emulsions were mixed with 1.0 ml of additive solution dissolved in 25 mmol/l NaCl in test tubes (12 mm $\phi \times 100$ mm) with a Vortex mixer (Scientific Industries, N.Y., USA), and the tubes were then kept at 30°C in a Taiyo Kagaku Kogyo TAH-1G hot dry bath (Tokyo). The final NaCl concentration was 12.5 mmol/l. At appropriate intervals, 0.5 ml aliquots of the mixture were sampled after uniform redispersion of emulsions through gentle mixing and diluted with 2.5 ml of 0.1% (w/v) sodium dodecyl sulfate (SDS). The diluent was then further diluted

In the second case, referred to as condition B, 0.2 g of ethyl oleate and 2.8 ml of 0.02% (w/v) Tween 20 were placed in test tubes (12 mm $\phi \times 100$ mm) and homogenized for 1 min at graduation 70 (corresponding to about 25,000 rpm) with a Physcotron NS-50 (Nichion Irika, Tokyo). The median diameter of the emulsions was about 11 µm. The prepared emulsions (0.1 ml) were mixed in the test tubes with 0.9 ml of an additive solution dissolved in 10 mmol/l NaCl and were then kept at 30°C in a heat-block bath. Seven mixtures or more were prepared to assess the stability of the emulsions. The final NaCl concentration was 9 mmol/l under these conditions. After a given period, 5.0 ml of 0.1% (w/v) SDS solution was added to each tube to dilute the emulsions, and the absorbance of the diluent at 500 nm was measured with a spectrophotometer.

The measurements were carried out at least in triplicate, usually six times, for each storage time, and the results were averaged. The coefficients of variation were in most cases within 2%.

Surface potential of emulsion droplets Aliquots of the emulsions prepared under condition A or B were diluted with a polymer solution of a given concentration, and the surface potential of at least 40 droplets was measured at room temperature with a Microtech Nichion ξ -potentiometer (Tokyo). The applied voltage was 50 V. The viscosity of the continuous phase was measured using a Ubbelohde type viscometer. The dielectric constant of the phase was assumed to be the same as that of water.

Surface and interfacial tensions The surface tensions of pullulan, maltodextrin, gum arabic and sodium caseinate solutions of different concentrations were measured at 30°C with a Kyowa Kaimen Kagaku CBVP-A3 surface tensiometer (Tokyo). The interfacial tensions between ethyl oleate and the additive solutions were also measured with the surface tensiometer.

1.2

1.0

0.8

Results

Effects of addition of various polymers on stability Figure 1 shows transient changes in absorbance at 500 nm of emulsions when various additives were added to the emulsions at 1.0% (w/v). Emulsions prepared under condition A were used in the experiments. The addition of neutral polymers such as pullulan PI-20, Dextran T-40 and maltodextrin destabilized the emulsions. On the other hand, the addition of polyelectrolytes, with the exception of SP-Dextran and gum arabic, stabilized the emulsions. Although gum arabic is used as a stabilizer (Nakamura, 1986), it destabilized the emulsions at the concentration used. Glucose, a small nonelectrolyte, showed no significant effect on the stability.

Effects of molecular weight of neutral polymer on stability Figure 1a suggests that neutral polymers of higher molecular weight destabilized the emulsions to a greater extent. To confirm this suggestion, emulsion stability was observed using dextrans and pullulans of different molecular weights (Fig. 2). In these experiments, emulsions prepared under condition A were used with polymers at concentrations of 1.0% (w/v). In a series of dextrans, the emulsions were destabilized as the molecular weight of dextran added became higher, but Dextran T-500 of the highest molecular weight examined did not greatly destabilize the emulsion. There were no marked effects of the molecular weight of pullulans on stability. This may have been due to the small differences in molecular weight among pullulans used compared with dextrans.

Effects of concentration of pullulan on stability As mentioned above, the concentration of an entrapping agent increased with the progress of dehydration of O/W emulsions in lipid encapsulation in a powdery matrix of the entrapping agent, and the stability of the emulsions was an important factor for successful encapsulation. Pullulan was used as an entrapping agent at the initial concentration of 15% (Imagi et



(a)

Absorbance at 500nm Â Ŷ 0.6 \diamond ☆ 0.4 0.2 4 8 0 2 6 Time [h]

Fig. 1. Effects of some additives on the stability of O/W emulsions at 30°C. The additives used were (a) glucose (\triangle), pullulan PI-20 (\bigcirc), Dextran T-40 (\Box) and maltodextrin (☉), and (b) SP-Dextran (☉), DEAE-Dextran (□), sodium caseinate (\Diamond), egg albumin (\triangle) and gum arabic (\triangledown). The concentration of each additive was 1.0% (w/v). Closed circles (●) indicate that no additive was added.

Fig. 2. Effects of molecular weight of Dextran and pullulan on the stability of O/W emulsions at 30°C. Open symbols, \triangle , \Box , \bigcirc and \Diamond , represent Dextran T-10, T-40, T-70 and T-500, respectively. Closed symbols, ▲, ■ and ◆, represent pullulans with number-averaged molecular weights of 6.5×104, 9.2× 10^4 and about 2×10^5 , respectively. The concentration of each polymer was 1.0% (w/v). Closed circle (\bullet) is the same as in Fig. 1.



Fig. 3. Effects of concentration of pullulan PI-20 on the stability of O/W emulsions at 30°C. The concentration of pullulan PI-20 was 0% (w/w) (\bullet), 1.0% (\triangle), 2.0% (\Box), 5.0% (\bigcirc) and 20% (\diamondsuit).



Fig. 4. Dependency of the stability of O/W emulsions on the concentration of pullulan PI-20 at 30°C. The stability was assessed by $(A_{120} - A_0)/A_0$, where A_0 and A_{120} were absorbances at 500 nm of the diluted emulsions at time t = 0 and t = 120 min, respectively.

al., 1992). Therefore, the stability of the emulsions was examined at various concentrations of pullulan PI-20. In these experiments, emulsions were prepared under condition B to estimate stability over short storage periods. Some of the results are illustrated in Fig. 3. Emulsions were destabilized as the concentration increased up to 5% (w/w). However, the stability of the emulsions at 20% pullulan was almost the same as that at 0%. To show the effect of the concentration on the stability, we calculated the values of $(A_{120} - A_0)/A_0$, where A_0 and A_{120} were the absorbance at time t=0 and t=120 min, respectively, as an indication of stability. The values are plotted against the concentration of pullulan PI-20 in Fig. 4. As shown in the figure, when the concentration of pullulan added was below 0.5% (w/w), there was no effect on the stability. At concentrations of 1 to 7% (w/w), the emulsions were greatly destabilized. At higher concentrations, they were again stabilized to a similar extent as in the case where no pullulan was added. This cannot be explained solely by considering the viscosity of the continuous phase because the



Fig. 5. ζ -Potential of emulsion particles at various concentrations of pullulan PI-20. The ζ -potential was measured at room temperature. The error bars indicate 95% confidence level.

Table 1. ζ -Potential of emulsion particles in 1.0% (w/v) polymer solutions.

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Additive	ζ-Potential [mV]
Pullulan PI-20	-41.6±2.1
Sodium caseinate	-42.9 ± 1.1
DEAE-Dextran	$61.2{\pm}2.8$
SP-Dextran	$-62.7{\pm}2.9$

Mean±95% confidence level.

viscosity increased as the concentration of pullulan increased, as shown later.

z-Potential of emulsion droplets The surface potential significantly affects the stability of emulsions. As a possible explanation for the results shown above, the surface potential of oil droplets may have changed with the concentration of polymer. Therefore, we measured the ξ -potentials of droplets at different pullulan concentrations (Fig. 5). The results shown in the figure were obtained using emulsions prepared under condition B. The potential was independent of the pullulan concentration up to 3% (w/w). The potential at higher concentrations could not be measured because of the high viscosity of the pullulan solution. The ξ -potentials of droplets were also measured for the other additives at the concentration of 1.0% (w/v) and are listed in Table 1. Emulsions prepared under condition A were used for these experiments. Addition of polyelectrolytes affected the ζ potentials of the droplets. As shown in Fig. 1, DEAE-Dextran stabilized the emulsions. This could be explained by the high absolute surface potential. However, SP-Dextran with almost the same absolute potential as DEAE-Dextran did not stabilize the emulsions. There are various possible explanations for these findings such as differences in viscosity and/or molecular weight, but it was not possible to draw any definite conclusions from the present results.

Surface and interfacial tensions Adsorption of polymers affects the stability of emulsions, and the interfacial viscosity plays a significant role in stability (Courthaudon *et al.*, 1991). To estimate the adsorption of some polymers on the interface, we measured the surface and interfacial tensions at various concentrations. As shown in Fig. 6, polyelectrolytes, sodium caseinate and gum arabic, seemed to be adsorbed at the interface, while neutral pullulan and maltodextrin were not. Therefore, stabilization of emulsions by sodium



Fig. 6. Surface and interfacial tensions at various concentrations of some polymers at 30°C. Polymers used were pullulan PI-20 (○, ●), maltodextrin (□, ■), sodium caseinate (△, ▲) and gum arabic (◇, ♦). Open symbols represent the surface tensions. Closed ones represent the interfacial tensions between water and ethyl oleate phases.



Fig. 7. Dependencies of viscosity (\bigcirc) and density (\triangle) on the concentration of pullulan PI-20 at 30°C.

caseinate, shown in Fig. 1b, would be ascribed to its adsorption (Dickinson, 1992). However, the weak destabilization by gum arabic could not be explained by adsorption at the interface. The bridging effect may take part in the destabilization.

Viscosity of pullulan solution The viscosity of the continuous phase would play an important role in stability, and emulsions would be more stabilized at higher viscosity. The viscosity of pullulan PI-20 solution was measured at various concentrations (Fig. 7). At higher concentrations, the viscosity increased.

Discussion

As mentioned above, the addition of a neutral polymer to the emulsions showed a peculiar effect on the stability, as shown in Fig. 4. To explain these phenomena, the depletion effect was incorporated in our previous model (Adachi *et al.*, 1992).



Fig. 8. (a) Schematic representation of the depletion effect and (b) distribution of force between two contiguous particles. The solid and broken curves represent the distributions of force when the depletion effect was ignored and was taken into consideration, respectively.

As shown schematically in Fig. 8a, there is a region where macromolecules cannot penetrate when emulsion particles approach each other. This would result in a difference in osmotic pressure between the region and the bulk phase, and the particles would tend to coalesce; this is called the depletion effect.

Let us consider two emulsion particles with the same diameter D approaching each other. There would be a potential barrier between the particles. Differentiation of the potential with respect to the separation x gives force, which can be estimated according to the DLVO theory (Verway & Overbeek, 1948). The points where electrostatic repulsion is in balance with the van der Waals-London attraction are designated by x_1 and x_2 (Fig. 8b). When a representative length L of a polymer is shorter than x_2 and the separation xis also less than x_2 , an attractive force operates on the coalescing particle. The distribution of the force in such a case would be expressed by the broken curve of Fig. 8b. The point where the force is zero is designated by x_1' .

By consideration of the depletion effect, the balance of forces operating on the particle is formulated by Eq. (1).

$$\frac{\pi}{6}D^3\rho_{\rm s}\frac{\mathrm{d}u}{\mathrm{d}t} = \frac{1}{4}\varepsilon D\psi_0^2 \frac{\varkappa \exp(-\varkappa x)}{1 + \exp(-\varkappa x)} - \frac{AD}{24\varkappa^2} - 3\pi D\eta u$$
$$-\frac{\pi}{6}D^3(\rho_{\rm f} - \rho_{\rm s})\alpha - \Pi \frac{\pi}{4}D^2 \left[2\frac{L-\varkappa}{D} - \left(\frac{L-\varkappa}{D}\right)^2\right](1)$$

where A is Hamaker's constant, t is the time, u is the migration velocity of the particle, Π is the osmotic pressure, α is the acceleration, ε is the dielectric constant of the dispersion medium, η is the viscosity of the medium, κ is Debye's factor, $\rho_{\rm f}$ is the density of the medium, $\rho_{\rm s}$ is the density of the particle, and ψ_0 is the surface potential. The

Table 2. Parameters used in calculation.

Hamaker constant A	10 ⁻²⁰ J
Particle diameter D	$1.2 \times 10^{-5} \text{ m}$
Length of polymer L	3.0×10⁻ ⁸ m
Molecular weight of polymer M	2.0×10 ⁴
Temperature T	303 K
Acceleration α	0 m/s ²
Dielectric constant of dispersion medium	ε 6.8×10 ⁻¹⁰ F/m
Debye factor \varkappa	$1.0 \times 10^8 \text{ m}^{-1}$
Density of particle ρ_s	970 kg/m³
Surface potential ϕ_0	$-3.66 \times 10^{-2} \text{ V}$

direction of repulsion is set to be positive for distance, force, and velocity. The first term on the right side represents electrostatic repulsion, and the second is the van der Waals-London attraction. The third term represents the frictional force by Stokes' law, and the fourth represents the buoyancy. The force by the depletion effect is expressed by the fifth term on the right side. Π is given by

$$\Pi = CRT \ (x \le L) \tag{2a}$$
$$= 0 \ (x > L) \tag{2b}$$

where C is the concentration of polymer, R is the gas constant, and T is the absolute temperature. The velocity u is defined as follows:

$$u = dx/dt$$
 (3)

Because the van der Waals-London attraction is very large inside x_1 or x_1' , the particle that arrives at x_1 or x_1' can coalesce with another particle. Therefore, the stability can be assessed by the energy needed for the particle to migrate from x_2 to x_1 or x_1' . For the initial conditions for Eqs. (1) and (3), the following equation is considered:

$$t=0, x=x_2; \frac{\pi}{12}D^3\rho_{\rm s}u^2=akT$$
 (u<0) (4)

where k is the Boltzmann constant. Equation (4) indicates that a particle located at x_2 gains kinetic energy at a times that of molecule kT at time t=0. The stability of the emulsions can be assessed by the a value at which the velocity of the particle becomes zero at x_1 or x_1' . The a value is denoted by a_c . The larger a_c value means that the emulsions are more stable.

Based on the above model, the effects of polymer concentration on the stability of the emulsions were examined. The parameters used are listed in Table 2. The buoyancy was excluded from consideration, that is, $\alpha = 0$. The values of Hamaker's constant and Debye's factor, which are difficult to evaluate experimentally, were assumed as shown in the table within reasonable ranges. The molecular weight and the representative length of the polymer were assumed to be $2 \times$ 10^4 and 30 nm, respectively.

Figure 9 shows the dependency of the a_c value on the concentration of the polymer. Curve B represents the dependency where the viscosity of the dispersion medium was assumed to be constant and to be that of water. In this case, an increase in the polymer concentration decreased the a_c value due to attractive force by the depletion effect. When the depletion effect was ignored and the change in the viscosity was considered (curve C in Fig. 9), the a_c value increased in



Fig. 9. Dependency of a_c on the polymer concentration. Curve A represents the dependency where both the depletion effect and the change in the viscosity were taken into consideration. Curve B represents the dependecy obtained under the assumption that the viscosity of the dispersion medium was independent of the concentration. Curve C represent the dependency where the depletion effect was neglected and changes in viscosity were considered.

parallel with the concentration. This was due to the increase in viscosity of the dispersion medium. The dependency of the a_c value on the concentration which was estimated by considering both the depletion effect and the change in viscosity with concentration is shown by curve A in Fig. 9. At low concentrations, the change in the viscosity was not a significant factor affecting the stability or the force by the depletion effect destabilized emulsions; that is, the a_c value decreased with concentration. At high concentrations, the frictional force was a predominant factor in stability and the a_c value became large. This indicates that the emulsions are stabilized at high concentrations.

The stability of emulsions was assessed by the $(A_{120} - A_0)/$ A_0 value in the experiments (Fig. 4) and by the a_c value in the above model (Fig. 9). Because the meanings of the values would not always be the same, only the comparison of the tendency between the values is possible. The experimentally observed dependency of the stability of emulsions on the concentration of polymer, shown in Fig. 4, could be expressed by the above model, although the range of concentrations where the destabilization of emulsions occurred was different. The difference would be due to some assumptions made in the calculation of the a_c value by the model. The model showed that both the frictional force by the dispersion medium and the attractive force of the depletion effect would play important roles in determining the stability of emulsions as well as the electrostatic repulsion and the van der Waals-London attraction when the dispersion medium contains a neutral polymer.

References

- Adachi, S., Imagi, J. and Matsuno, R. (1992). Model for estimation of the stability of emulsions in a cream layer. *Biosci. Biotech. Biochem.*, 56, 495-498.
- Asakura, S. and Oosawa, F. (1954). On interaction between two bodies immersed in a solution of macromolecules. J. Chem. Phys., 22, 1255-1256.

- Courthaudon, J.-L., Dickinson, E. and Dalgleish, D.G. (1991). Competitive adsorption of β -casein and nonionic surfactants in oil-in-water emulsions. J. Colloid Interface Sci., 145, 390–395.
- Courthaudon, J.-L., Dickinson, E., Matsumura, Y. and Williams, A. (1991). Influence of emulsifier on the competitive adsorption of whey proteins in emulsions. *Food Struct.*, **10**, 109-115.
- Dickinson, E. (1992). Structure and composition of adsorbed protein layers and the relationship to emulsion stability. J. Chem. Soc. Faraday Trans., 88, 2973-2983.
- Feigin, R.I. and Napper, D.H. (1980). Depletion stabilization and depletion flocculation. J. Colloid Interface Sci., 75, 525-541.
- Fleer, G.J. and Scheutjens, J.M.H.M. (1987). Effect of adsorbing and nonadsorbing polymer on the interaction between colloidal particles. *Croatica Chem. Acta*, **60**, 477-494.
- Imagi, J., Kako, N., Nakanishi, K. and Matsuno, R. (1990). Entrapment of liquid lipids in matrixes of saccharides. J. Food Eng., 12, 207-222.
- Imagi, J., Yamanouchi, T., Okada, K., Tanimoto, M. and Matsuno, R. (1992). Properties of agents that effectively entrap liquid lipids. *Biosci. Biotech. Biochem.*, 56, 477-480.
- Nakamura, M. (1986). Stabilization of emulsion by natural polymer: study of gum arabic. Yukagaku, 35, 554-560 (in Japanese).
- Pearce, K.N. and Kinsella, J.E. (1978). Emulsifying properties of proteins: evaluation of a turbidimetric technique. J. Agric. Food Chem., 26, 716-723.
- Verway, E.J.W. and Overbeek, J.Th.G. (1948). "Theory of the Stability of Lyophobic Colloids." Elsevier, Amsterdam.