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Role of Macromolecules in Emulsion Stability with Special Reference to Pepsin as Emulsifier

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Role of pepsin as emulsifier in the stability of Nujol-in-water system has been studied. The flocculation studies were carried out haemocytometrically at different pH values, different amounts of electrolytes and different amounts of emulsifier itself. The zeta potential measurements were carried out at different pH values and varying amounts of electrolytes. In the light of the D. V. O. theory the interaction energy profiles have been drawn by summing up the repulsion and attraction energies. The high energy barriers obtained obviate the possibility of the occurrence of the flocculation in the primary minima. It appears, therefore, that the reversible flocculation occurs in the secondary minima which are about 2 to 4 kT deep. Therefore, assuming a singlet-doublet equilibrium degrees of aggregations have been calculated. In order to explain the observed values of degrees of aggregations, A (van der Waals constant) should be $\approx 5.97 \times 10^{-13}$ erg. This value has been obtained by an indirect approach to fit the aggregation data. In case of Al(III) and Th(IV), however, charge reversal takes place which has been explained by a model picture. Charge densities and binding parameters have also been calculated.

It has been reported by previous workers that most of the macromolecules are good emulsifying agents, which increase the emulsion stability many times. Around the oil droplets they form adsorbed layers which by virture of their visco-elastic nature prevent coagulation. Rehbinder and Trapeznikov(1) observed that oil in water systems are remarkably stable because of the viscoelastic properties of the interfacial films. Similar conclusions were drawn later on by King,(2) who showed that when the globules are surrounded by a complete sheath of the emulsifying agent, the electrical stability factor becomes relatively unimportant and then emulsion resembles a protective colloid.

Recently Srivastava(3) studied the role of viscoelastic characteristic of the BSA film in the coagulation of the petroleum ether-in-water emulsions. The same systems were studied by the same worker(4) also in the light of potential energy of interaction.

The total interaction between droplets comprises the electrostatic repulsion caused by the electrical double layer around the droplets and the van der Waals attraction.(5) The repulsive energy mainly depends upon the ions adsorbed on the surface from the dispersion medium and the surface potential. The van der Waals attraction on the other hand finds its origin in the London dispersion forces. Such interactions have been reported by Derjaguin,(6,7) Verwey and Overbeek(8) under their well known theory of colloid stability, the so-called D. V. O. theory. It will, however, be found that only a little work has been done in the field of macromolecular stabilized emulsions with regard to this theory.

In the present work an attempt has been made to study quantitatively the stability of Nujol (weighted by chlorobenzene)-in-water emulsions stabilized by the macromolecule, pepsin. Using D. V. O. theory interaction energies between the coated droplets have been calculated as a function of surface potentials and ionic strengths. From the interaction energy data available the charge densities in the Stern and Gouy layers and binding parameters have been evaluated. Also, since in the systems investigated flocculation takes place in the secondary minima,(9-11) coagulation results have been given in terms of degrees of aggregations

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1) Rehbinder and Trapeznikov, *Acta Phys. Chim., URSS*, **62**, 275 (1938).

2) A. King, *Trans. Faraday Soc.*, **46**, 252 (1950).

3) S. N. Srivastava, *J. Indian Chem. Soc.*, **41**, 279 (1964).

4) S. N. Srivastava, *Indian J. Chem.*, **3**, 376 (1965).

5) J. Th. G. Overbeek, cited in colloid science, Vol. I, by H. R. Kruyt, Elsevier publishing company, Amsterdam (1952), p. 245.

6) B. Derjaguin, *Trans. Faraday Soc.*, **36**, 203, 730 (1940).

7) B. Derjaguin and L. D. Landau, *Acta Phys. Chim., URSS*, **14**, 633 (1941).

8) E. J. W. Verwey and J. Th. G. Overbeek, "Theory of Stability of Lyophobic Colloids," Elsevier Publishing Co., Amstardam (1948).

9) J. N. Schenkel and J. A. Kitchener, *Trans. Faraday Soc.*, **56**, 161 (1960).

10) M. V. Tempel, *cf.* "Stability of Oil-in-water Emulsions," Rubber stichting, oostsingel, 178, Delft (Holland).

11) S. N. Srivastava, and D. A. Haydon, *Trans. Faraday Soc.*, **60**, 971 (1964).

which have been determined both experimentally and theoretically. With the help of the two sets of aggregation data it was possible to calculate the van der Waals constant of the coated drops.

Theoretical

In the frame work of the D. V. O. theory the interaction energy of the colloidal particles is given by $V=V_R+V_A$, where V_A=attraction energy, V_R=repulsive energy and V=sum of the repulsion and attraction energies. The double layer repulsion V_R has been calculated from an approximate expression given by Derjaguin[12] and Kussakov

$$V_R = \frac{\varepsilon a \psi^{\circ 2}}{2} \ln (1 + e^{-\kappa H}) \tag{1}$$

where ψ°=the surface potential (assumed equal to the electro-kinetic potential for the present system having $\kappa a \gg 1$), ε is the dielectric constant in the double layer (assumed equal to the bulk value, 80, the dielectric constant of H_2O); a the particle radius, H the interparticle distance and κ the Debye-Hückel parameter. The validity of Eq. (1) for the present system is justified by the large value of κa ranging between 0.38×10^2 to 1.5×10^2. V_A is calculated from the following Eq. (2)

$$V_A = \frac{-Aa}{12H} \tag{2}$$

where A is the van der Waals constant, which is taken equal to 1×10^{-13} erg as used by van den Tempel[10] for initial calculations, thus the interaction energy V can be represented as

$$V = \frac{\varepsilon a \psi^{\circ 2}}{2} \ln (1 + e^{-\kappa H}) - \frac{Aa}{12H} \tag{3}$$

Therefore, knowing V from Eq. (3) the number of particles in a shell a d_s round a central particle in the emulsion system, can be calculated from

$$4\pi n_0 a^3 s^2 \exp(-V/\boldsymbol{k}T) d_s$$

where n_0 is the number of droplets per unit volume of the suspension and $s=2+(H/a)$. On integrating the above expression D, the degree of aggregation or the total number of particles associated with a given central particle is obtained

$$D = 4\pi a^3 n_0 \int s^2 \exp(-V/\boldsymbol{k}T) d_s \tag{4}$$

The integration of the above expression is evaluated graphically. If the ionic strength of the system is increased by addition of uni- or polyvalent electrolytes, the compression of the double layer takes place, causing flocculation. In case of the reversible or secondary minimum flocculation of the present investigation as depicted in (Fig. 1) a singlet-doublet equilibrium temporarily exists in the system. The extent of flocculation in the system can be calculated from the formula

$$D = \frac{\text{number of doublets}}{\text{number of doublets} + \text{number of singlets}} \tag{5}$$

where D is the number of droplets associated with a specified particle *i. e.* the degree of aggregation.

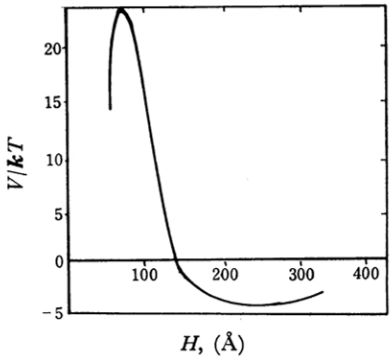

Fig. 1. It shows the occurrence of secondary minimum in large particles.

Charge Density and Binding Parameters. The charge density in the Gouy layer for the large particles can be calculated by the formula

$$\sigma_2 = \frac{\varepsilon \xi \kappa}{4\pi}$$

where ε=dielectric constant, ξ=zeta potential, κ=Debye Hückel parameter. This equation is valid for small values of ξ-potentials and low values of κa only. Therefore, the correction has to be applied for the large values of κa and ξ-potentials. Then charge density will be given by

$$\sigma_2 = \frac{\varepsilon\kappa}{4\pi} \cdot \frac{\boldsymbol{k}T}{e} \cdot 2^{1/2} \left\{ \frac{\exp(Z_-(e\xi/\boldsymbol{k}T))-1}{Z_-(Z_-+Z_-)} + \frac{\exp(-Z_+(e\xi/\boldsymbol{k}T))-1}{Z_+(Z_++Z_-)} \right\}^{1/2} \tag{6}$$

where Z_+ and Z_- are valencies of positive and negative ions, $\boldsymbol{k}$ is the Boltzman's constant, e= elementary charge. With the help of the charge density in the Stern layer, σ_s the binding parameters have also approximately been calculated with the following treatment. On adding small quantities of foreign ions (which in effect will not change the strength), capable of being adsorbed or bound to the protien covering the emulsion droplets, the charge density will change and will be given by the Stern's equation

$$\sigma_s = \frac{N_1 e v}{1+(55.6/c)\exp(\Delta G/\boldsymbol{k}T)}$$

where c is the molar concentration of the added salt, N_1 the binding sites per cm^2 and $-\Delta G$ is the free energy of adsorption per molecule. This can be put in the form[13,14]

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[12] B. Derjaguin, and M. Kussakov, *Acta Phys. Chim., URSS.,* **10**, 25, 153 (1939).

[13] D. J. Wilkins *et al., J. Theoret. Biol.,* **2**, 173 186 (1962).

[14] R. H. Ottewill, *et al., Trans. Faraday Soc.,* **56**, 854 (1960).

$$\left.\begin{aligned} \sigma_s &= \frac{k_1 c}{1+k_2 c} \\ \text{where} & \\ k_2 &= \frac{\exp(-\Delta G/\boldsymbol{k}T)}{55.6} \\ k_1 &= N_1 k_2 e v \end{aligned}\right\} \qquad (7)$$

These k_1 and k_2 are the adsorption constants which have been calculated and from these adsorption constants the binding sites available and free energy of adsorption have been evaluated. These constants can also be calculated from the treatment given by Srivastava[15] at the zero point of charge, which finally yields the following equations

$$\frac{1}{c} = \frac{4\pi e v N_1 k_2}{\kappa \varepsilon \psi^\circ_\delta} - k_2 \qquad (8)$$

$$\left(\frac{\mathrm{d}\psi\delta}{\mathrm{d}\ln c}\right)\psi^\circ_{\delta=0} = \left(\frac{\kappa\varepsilon\psi_\delta}{4\pi e v K_1} - 1\right)\psi^\circ_\delta \qquad (9)$$

where v is the valency and $(\mathrm{d}\psi\delta/\mathrm{d}\ln c)\psi^\circ_{\delta=0}$ is the slope of the log c-zeta potential curve (assuming $\psi^\circ_\delta = \xi^\circ$), c is the concentration of electrolyte at the zero point of charge.

Experimental Procedure

The pepsin obtained was of M & B Co., This was further crystallised and dried. The Nujol and chlorobenzene were well purified before use. The glass apparatus used was all pyrex and the water was twice distilled. Other materials used were of analar grade and were used without further purification.

Emulsion

The emulsion was prepared by suspending 4% by volume of oil in aqueous solution of 0.01% pepsin containing 0.01% KCl. The emulsion was first hand shaken for about 1/2 hr and finally homogenized well in a stainless steel homogenizer (Schaar scientific Co., Chicago). The freshly prepared emulsion was kept for about half an hour and then the creamed layer was skimmed off. The sampling was done under identical conditions.

The flocculation of particles was observed by counting the number of oil droplets haemocytometrically, using an improved double neubauer model. About 600 globules were counted each time in 84 squares of the graticule fitted in the eyepiece (each square corresponds to a volume of 7.74×10^{-7} m*l*) with a hand tally counter (Erma, Tokyo, Japan) under a Olympus microscope using 15× 100 times magnification. A duplicate counting was always carried out with another sample and the mean was taken. The results were reproducible to 5%.

The pH determinations were carried out at room temperature on the Cambridge pH meter with an accuracy of 0.1 unit. Under normal condition the pH of the emulsion was 6.0±0.2.

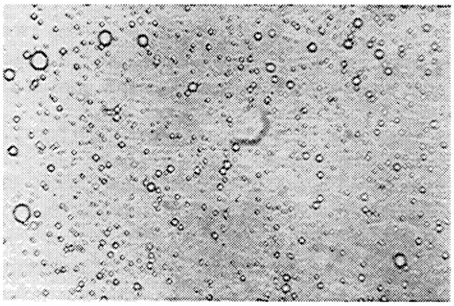

Fig. 2. Microphotograph of the emulsion droplets with 2400 times magnification.

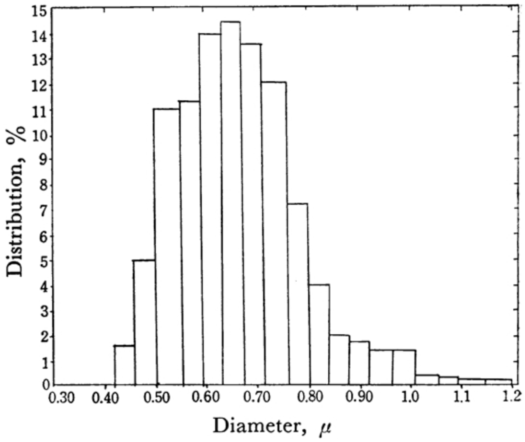

Fig. 3. Size distribution curve of the system.

Gallenkamp's direct reading photoelectric colorimeter No. 3615 was used in measuring the optical densities.

For the size of the drops the microphotograph was taken (Fig. 2) with olympus camera. The size of about 500 drops was measured and a size frequency curve was plotted (Fig. 3). The average diameter of the droplets was found to be 0.65 μ.

The electrophoretic measurements were carried out by moving boundary method at a constant current and the potential across the electrodes was measured by V. T. V. M. The zeta potential from the mobility data was calculated by using Overbeek equations (10) and (11) for symmetrical and unsymmetrical electrolytes

$$u = \frac{\varepsilon\xi E}{6\pi\eta}\left[f_1(\kappa a) - Z^2\left(\frac{e\xi}{\boldsymbol{k}T}\right)^2 f_3(\kappa a) - \frac{\rho_+ + \rho_-}{2e}\cdot\frac{\varepsilon\boldsymbol{k}T}{6\pi\eta e}\left(\frac{e\xi}{\boldsymbol{k}T}\right)^2 f_4(\kappa a)\right] \qquad (10)$$

$$u = \frac{\varepsilon\xi E}{6\pi\eta}\left[f_1(\kappa a) - (Z_- - Z_+)\frac{e\xi}{\boldsymbol{k}T} f_2(\kappa a) - \frac{Z_+\rho_+ + Z_-\rho_-}{(Z_+ + Z_-)e}\cdot\frac{\varepsilon\boldsymbol{k}T}{6\pi\eta e}\left(\frac{e\xi}{\boldsymbol{k}T}\right)^2 f_4(\kappa a)\right] \qquad (11)$$

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[15] S. N. Srivastava, *J. Indian Chem. Soc.*, **41**, 203 (1964).

Table 1

Concentration of pepsin %	I Addition of pepsin before dispersion		II Addition of pepsin after dispersion	
	Initial No. of drops/ml	Half lives hr	Initial No. of drops/ml	Half lives hr
0.01	10.9×10^8	108	10.3×10^8	105
0.05	7.87×10^8	86	10.3×10^8	97
0.10	7.29×10^8	72	10.3×10^8	108
0.15	6.85×10^8	63	10.3×10^8	92
0.20	6.57×10^8	24	10.3×10^8	90

where u=velosity of the boundary, $f_1(\kappa a)$ is identical with Henry factor, $f_2(\kappa a)$, $f_3(\kappa a)$ and $f_4(\kappa a)$ are correction terms tabulated by Overbeek.[16] ρ_+ and ρ_- are the friction constants for cations and anions respectively, which are calculated from the mobility of ions, η is the viscosity of the dispersion medium, $\boldsymbol{k}$ is the Boltzman constant, e is the electronic charge, Z_+ and Z_- are the valencies of cations and anions.

Results and Discussion

Effect of Pepsin Concentration. The coagulation of the emulsion was studied at five different concentrations of the pepsin to see its influence on stability and the emulsifier was added in two ways *i. e.* before the dispersion of oil and after the dispersion of the oil which had already been emulsified with an initial concentration of 0.01% of pepsin. The half lives are tabulated in Table 1.

In the former case the initial number of drops is reduced and the stability is decreased with an increasing concentration of pepsin perhaps due to desorption. In the latter case there is no appreciable change in the stability and therefore, there is no regularity in their half lives. The number of drops/ml of the former case has been plotted against the time (Fig. 4).

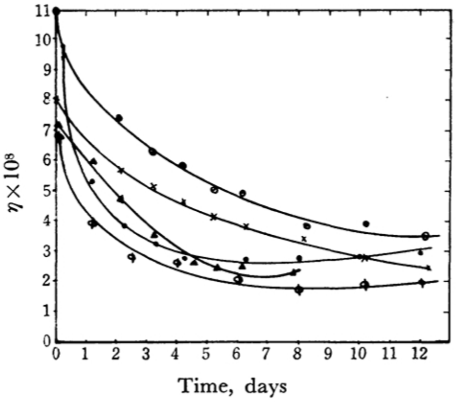

Fig. 4. Plots of time *vs.* number of particles/ml at (⊙) 0.01%, (×) 0.05%, (▲) 0.10%, (∅) 0.15%, (●) 0.20% concentration of pepsin using as emulsifier.

Effect of pH. As mentioned above the initial emulsion had pH 6.2. It was most stable at this pH and had a half life of 52 hr but on changing the pH both towards acidic and alkaline side, a decrease in half lives was observed (Fig. 5). The decrease was however, more rapid in the acidic side than in the alkaline side (Table 2). This may be because of the fact that the iso-electric point

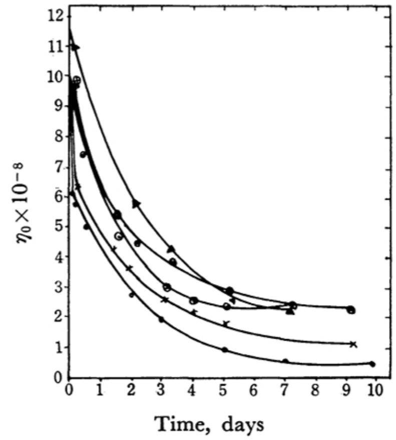

Fig. 5. Plots of time *vs.* number of particles/ml at different pH values (●) pH 3.1, (×) pH 4.3, (⊙) pH 5.6, (▲) pH 6.2, (⊗) 8.5.

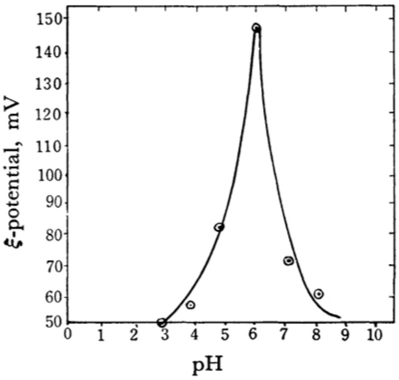

Fig. 6. Plots of pH *vs.* corresponding ξ-potential.

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[16] J. Th. G. Overbeek, "Advances in Colloid Science," Vol. III, p. 115.

(pH 2.8) of the emulsion lies towards the acidic side. At this pH as expected the flocculation is instantaneous. These results are also borne out by

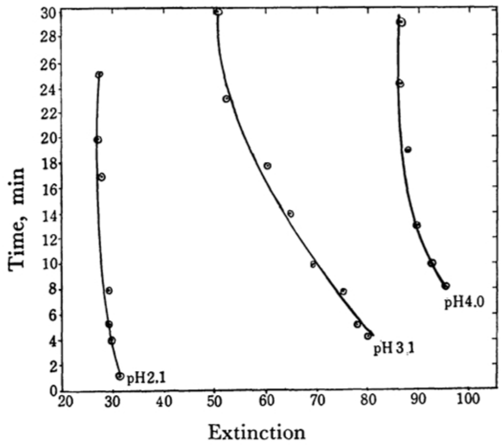


Fig. 7. Plots of extinction *vs.* time (min).

TABLE 2

pH	Half lives (determined haemocytometrically)
2.8 (iso-electric point)	Almost instantaneous flocculation
3.1	2.0 hr
4.3	10.0 hr
5.6	14.0 hr
6.2	52.0 hr
7.1	39.0 hr
8.5	32.0 hr

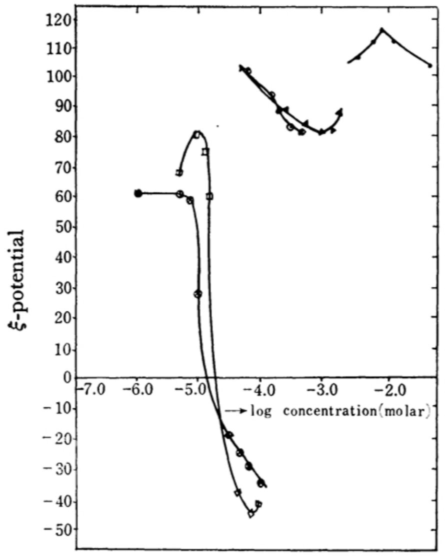


Fig. 8. Plots of log concentration (molar) *vs.* zeta potential.

(●) KCl (▲) $BaCl_2$ ⊙ $UO_2(NO_3)_2$
(□) $AlCl_3$ (⊕) $Th(NO_3)_4$

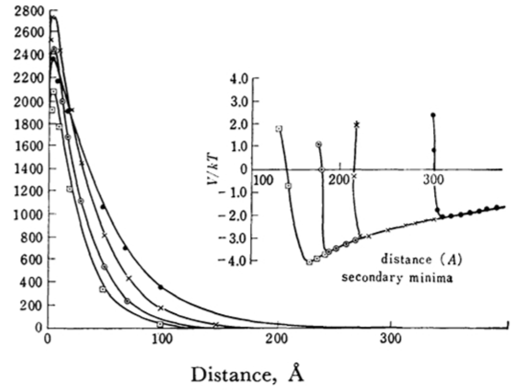


Fig. 9. Plots of distance (Å) and potential energy of interaction showing the heights of maxima in primary and secondary minima.
(●) 5×10^{-3} M KCl, (×) 1.0×10^{-2} M KCl
(⊙) 1.5×10^{-2} M KCl, (⊡) 2.0×10^{-2} M KCl

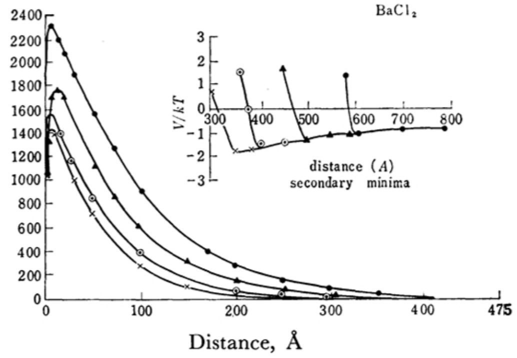


Fig. 10. Plots of distance and potential energy of interaction for $BaCl_2$ at different concentrations showing the heights of maxima in primary and secondary minima.
(●) 5×10^{-5} M, (▲) 2×10^{-4} M, (⊙) 6×10^{-4} M,
(×) 1×10^{-3} M

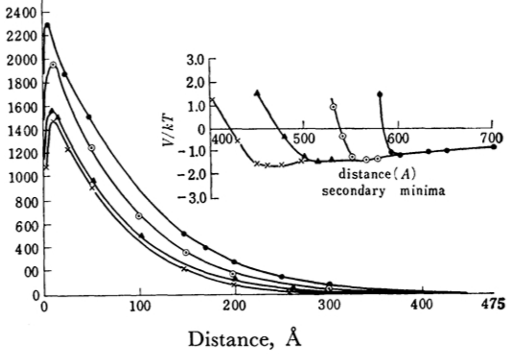
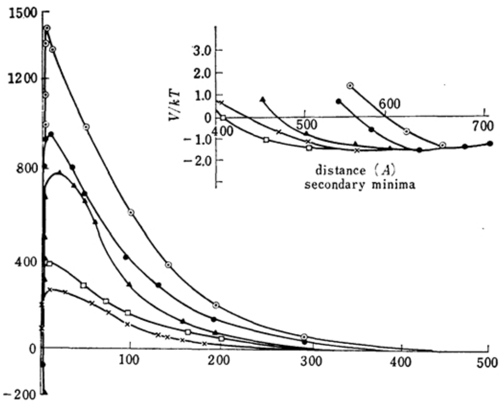


Fig. 11. Plots of distance and potential energy of interaction for $UO_2(NO_3)_2$ at different concentrations showing the hights of maxima in primary and secondary minima.
(●) 5×10^{-5} M, (⊙) 1.5×10^{-4} M, (▲) 3×10^{-4} M
(×) 4×10^{-4} M

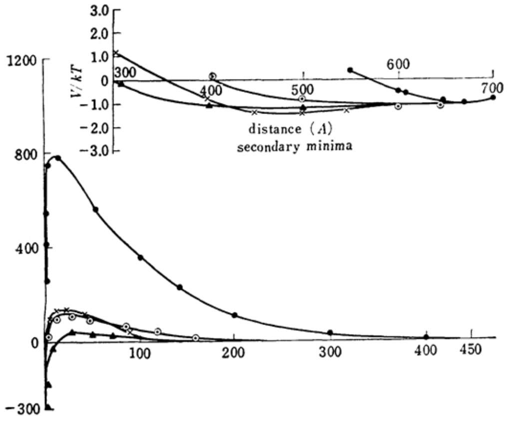
measurements of zeta potentials as a function of pH (Fig. 6). The effect of pH changes on emulsion stability has been further studied colorimetrically. The extinction *vs.* time graph is plotted (Fig. 7). The extinction in the acid side decreases with the decrease in pH, while in the alkaline side the extinction is always ∞, showing smaller degree of coalescence.

Influence of Electrolytes on the Zeta Potential. Changes in the ξ-potential by the addition of electrolytes have been studied. The initial charge of the emulsion was negative ($\xi = -147.4$ mV), so the flocculation was possible only by means of cations. A graph has been plotted (Fig. 8) between the zeta potentials and the log molar concentration of the added electrolytes, *viz*, KCl, $BaCl_2$, $UO_2(NO_3)_2$, $AlCl_3$, $Th(NO_3)_4$. The nature of these curves is identical with those observed by Rutgers[17] and Smet. First there is a slight increase in zeta potential with the addition of KCl after which a regular decrease is observed.[18,19] With bivalent cations the curves are normal but on the addition of higher concentration of $AlCl_3$ and $Th(NO_3)_4$ the reversal of charge takes place. These results of the variation in ξ-potential with the addition of electrolytes are in keeping with the flocculation results.



Distance, Å

Fig. 12. Plots of distance and potential energy of interaction for $AlCl_3$ at different concentrations showing the heights of maxima in primary and secondary minima.
(●) 5×10^{-6} M, (⊙) 1×10^{-5} M, (▲) 1.5×10^{-5} M, (×) 5×10^{-5} M, (□) 1×10^{-4} M

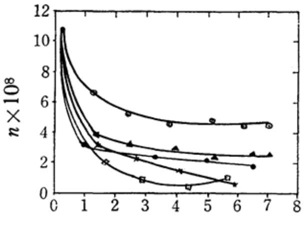


Distance, Å

Fig. 13. Plots of distance and potential energy of interaction for $Th(NO_3)_4$ at different concentrations showing the heights of maxima in primary and secondary minima.
(●) 1×10^{-6} M, (⊙) 1×10^{-5} M, (▲) 3×10^{-5} M, (×) 7×10^{-5} M

Interaction Energy Curves and Stability Factor. To have a clear insight into emulsion coagulation interaction energy curves have been plotted for different electrolyte contents using Eq. (3) and represented in (Figs. 9—13). From the figures it is obvious that the curves have got very high energy barriers showing a high degree of stability as well as high value of stability factor. The extent of the energy barriers varies with zeta potential and κ which depend on the electrolyte content. These maxima are, however, comparatively smaller in the case of tri- and tetravalent cations (Table 3) which appreciably reduce the zeta potential.

Flocculation in the Secondary Minima and Determination of van der Waals Constant. The high values of energy barriers obviate the possibility of occurrence of flocculation in the primary minima. Nevertheless, flocculation has been observed to occur in these systems at an appreciable rate. To account for this, flocculation has been assumed to occur in the secondary minima. In fact, the secondary minima obtained in the energy contours of Figs. 9—13 are upto 4 kT deep where the droplets are still separated 300 to 600 Å but



Time, hr

Fig. 14. Plots of time and number of monomers/ml (which are not flocculated) at different concentrations of KCl.
(●) 5×10^{-3} M, (⊙) 1×10^{-2} M, (▲) 1.5×10^{-2} M, (×) 2×10^{-2} M, (□) 2.5×10^{-2} M

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[17] A. J. Rutgers and M. DeSmet, *Trans. Faraday Soc.*, **41**, 758 (1945).

[18] H. R. Kruyt, *Kolloidzeitscher*, **22**, 81 (1918).

[19] Freundlich and Rona, *Sitzungsher, Preuss, Akad. wiss*, **23**, 397 (1920).

TABLE 3

Electrolyte concentration	Zeta potential mV	Height of maxima, kT	Interparticle distance at which interaction ($V=0$)	Depth of the secondary minima, kT
Initial emulsion	-147.4	4935	—	—
5×10^{-3} M KCl	-105.84	2396	303 Å	2.1
1×10^{-2} M KCl	-114.8	2738	215 Å	3.0
1.5×10^{-2} M KCl	-111.0	2470	175 Å	3.7
2.0×10^{-2} M KCl	-103.4	2079	138 Å	4.2
5×10^{-5} M $BaCl_2$	-101.6	2302	575 Å	1.1
2×10^{-4} M $BaCl_2$	-89.04	1779	472 Å	1.4
6×10^{-4} M $BaCl_2$	-85.2	1527	375 Å	1.8
1×10^{-3} M $BaCl_2$	-80.9	1429	305 Å	1.9
1.5×10^{-3} M $BaCl_2$	-81.9	1352	270 Å	2.1
2.0×10^{-3} M $BaCl_2$	-87.4	1553	250 Å	2.4
5×10^{-5} M $UO_2(NO_3)_2$	-101.6	2302	572 Å	1.1
1×10^{-4} M $UO_2(NO_3)_2$	-95.6	1988	516 Å	1.3
1.5×10^{-4} M $UO_2(NO_3)_2$	-93.3	1950	520 Å	1.3
2.0×10^{-4} M $UO_2(NO_3)_2$	-89.56	1779	470 Å	1.4
3.0×10^{-4} M $UO_2(NO_3)_2$	-86.0	1591	466 Å	1.5
4.0×10^{-4} M $UO_2(NO_3)_2$	-84.7	1522	418 Å	1.7
5×10^{-6} M $AlCl_3$	-66.8	936.6	545 Å	1.2
1×10^{-5} M $AlCl_3$	-81.8	1411.0	598 Å	1.0
1.25×10^{-5} M $AlCl_3$	-75.4	1204.0	595 Å	1.10
1.5×10^{-5} M $AlCl_3$	-59.4	743.5	450 Å	1.4
Charge reversal				
5×10^{-5} M $AlCl_3$	$+38.1$	260.0	440 Å	1.3
7×10^{-5} M $AlCl_3$	$+44.5$	377.0	462 Å	1.2
1×10^{-4} M $AlCl_3$	$+41.6$	321.0	410 Å	1.2
1×10^{-6} M $Th(NO_3)_4$	-61.5	784.0	548 Å	1.1
5×10^{-6} M $Th(NO_3)_4$	-61.5	774.0	548 Å	1.1
7×10^{-6} M $Th(NO_3)_4$	-60.5	757.0	548 Å	1.1
1×10^{-5} M $Th(NO_3)_4$	-27.6	110.6	396 Å	1.0
Charge reversal				
3×10^{-5} M $Th(NO_3)_4$	$+18.0$	38.8	297 Å	1.1
5×10^{-5} M $Th(NO_3)_4$	$+24.8$	76.5	346 Å	1.0
7×10^{-5} M $Th(NO_3)_4$	$+29.0$	125.0	336 Å	1.0
1×10^{-4} M $Th(NO_3)_4$	$+34.4$	195.0	340 Å	1.3

TABLE 4

Concentration of electrolyte	Value of A corresponding to $D\%$ observed	Mean value of A
5×10^{-5} M $BaCl_2$	6.4×10^{-13} erg	5.97×10^{-13} erg
6×10^{-4} M $BaCl_2$	4.3×10^{-13} erg	
5×10^{-5} M $UO_2(NO_3)_2$	6.25×10^{-13} erg	
1.5×10^{-4} M $UO_2(NO_3)_2$	5.4×10^{-13} erg	
4×10^{-4} M $UO_2(NO_3)_2$	4.5×10^{-13} erg	
1×10^{-5} M $AlCl_3$	7.0×10^{-13} erg	
1.5×10^{-5} M $AlCl_3$	6.2×10^{-13} erg	
1×10^{-5} M $Th(NO_3)_4$	6.5×10^{-13} erg	
1×10^{-6} M $Th(NO_3)_4$	7.1×10^{-13} erg	

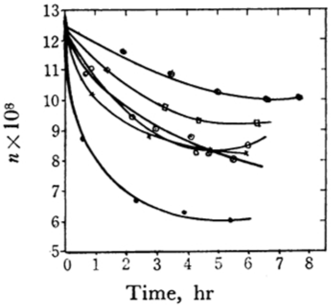

Fig. 15. Plots of time and number of monomers/ ml (which are not flocculated) at different concentrations of $BaCl_2$.
(⊕) 5×10^{-5} M, (⊡) 2×10^{-4} M, (○) 6×10^{-4} M, (×) 1×10^{-3} M, (⊙) 1.5×10^{-3} M, (●) 2×10^{-3} M

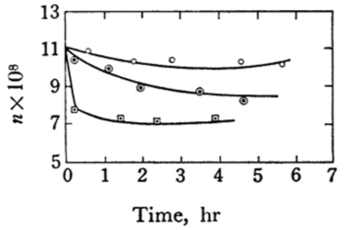

Fig. 16. Plots of time and number of monomers/ ml (which are not flocculated) at different concentrations of $UO_2(NO_3)_2$.
(⊙) 4×10^{-4} M, (⊡) 1.5×10^{-4} M, (○) 5×10^{-5} M

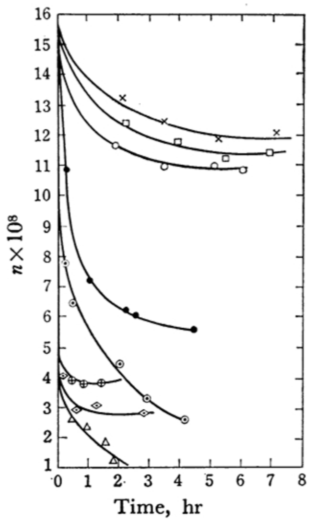

Fig. 17. Plots of time and number of monomers/ ml (which are not flocculated) at different concentrations of $AlCl_3$.
(⊙) 1.5×10^{-5} M, (●) 1.25×10^{-5} M, (○) 1×10^{-5} M, (×) 7.5×10^{-6} M, (□) 5×10^{-6} M
Charge reversed, (△) 5×10^{-5} M, (⊕) 7×10^{-5} M, (◈) 1×10^{-4} M

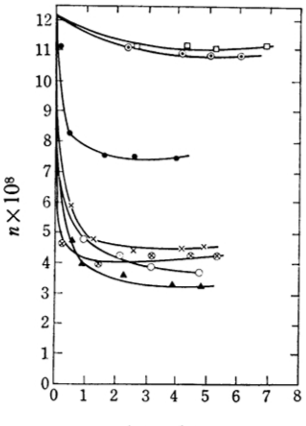

Fig. 18. Plots of time and number of monomers/ ml (which are not flocculated) at different concentrations of $Th(NO_3)_4$.
(⊡) 5×10^{-6} M, (⊙) 7×10^{-6} M, (●) 1×10^{-5} M
Charge reversed, (×) 3×10^{-5} M, (▲) 5×10^{-5} M, (○) 7×10^{-5} M, (⊕) 1×10^{-4} M

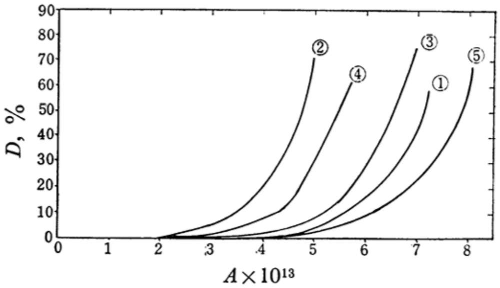

Fig. 19. Plots of van der Waals constant (A) and degree of aggregation (D%) at
① 5×10^{-5} M $BaCl_2$, 5×10^{-5} M $UO_2(NO_3)_2$
② 6×10^{-4} M $BaCl_2$, 1.5×10^{-5} M $AlCl_3$, 1×10^{-5} M $Th(NO_3)_4$
③ 1.5×10^{-4} M $UO_2(NO_3)_2$
④ 4×10^{-4} M $UO_2(NO_3)_2$
⑤ 1×10^{-5} M $AlCl_3$, 1×10^{-6} M $Th(NO_3)_4$

are flocculated. This reversible nature of flocculation is justified by the large size of particles with adsorbed pepsin. The depth of the secondary minima and the interparticle distance at zero point interaction ($V=0$) are tabulated in Table 3.

The flocculation sets a temporary equilibrium after a time $t>R^2/D'$ where R is the radius and D' is the diffusion constant (in the present case $t\approx20$ min) and a singlet-doublet equilibrium exists. For every sample the experimental degree of aggregation has been calculated with the help of Eq. (5) by counting the singlets and doublets. The number of monomers have been plotted against time (Figs. 14—18) showing the decrease of the particles in different concentrations of electrolytes. The degrees of aggregation have also been calculated theoretically at different van der Waals constants varying from 2×10^{-13} to 7×10^{-13} erg with the help of Eq. (4). A graph is plotted between van der Waals constant and D% (degree of aggregation), as shown in Fig. 19. From this

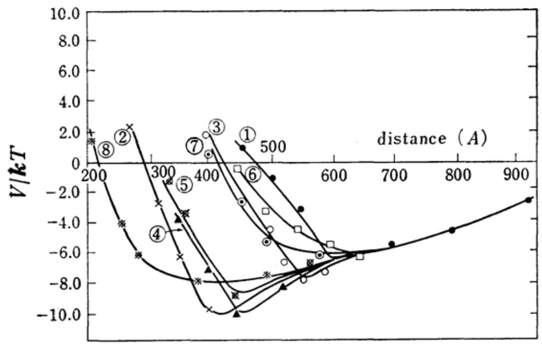

Fig. 20. Plots showing the depth of secondary minima taking van der Waals constant (A) as 5.97×10^{-13} erg at
① 5×10^{-5} M $BaCl_2$ and 5×10^{-5} M $UO_2(NO_3)_2$
② 6×10^{-4} M $BaCl_2$
③ 1.5×10^{-4} M $UO_2(NO_3)_2$
④ 4×10^{-4} M $UO_2(NO_3)_2$
⑤ 1.5×10^{-5} M $AlCl_3$
⑥ 1×10^{-5} M $AlCl_3$
⑦ 1×10^{-6} M $Th(NO_3)_4$
⑧ 1×10^{-5} M $Th(NO_3)_4$

graph the values of A corresponding to the observed values of D are noted and tabulated in Table 4. In this way a mean value of A for the present system 5.97×10^{-13} erg has been obtained.

For the calculations of the energy profiles (Figs. 9—13) a value of $A=1\times10^{-13}$ erg was chosen as suggested by van den Tempel (*loc. cit.*), the low value of the van der Waals constant being in agreement with the work of other authors[9,15] as well. This gave high energy barriers and secondary minima which were upto 4 kT deep. It could no doubt explain the reversible flocculation of the present work but it yielded theoretical values of degrees of aggregation which did not compare with the observed values. Therefore, secondary minima have been again plotted (Fig. 20) with the observed value of A, 5.97×10^{-13} erg. These are upto 10 kT deep and this is a reasonable depth for the secondary minimum flocculation to occur.

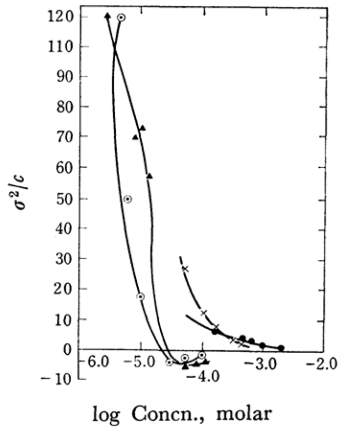

Fig. 21. Plots between log concentration (molar) and σ_2/c, charge density in Coulombs/M.

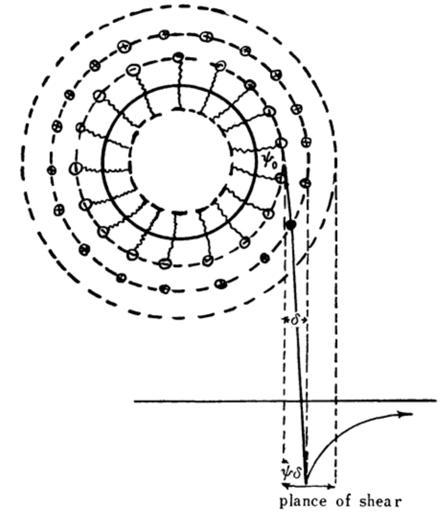

Fig. 22. Charge reversal model which shows that it is possible when n_c, the number of counter ions adsorbed per cm² from a bulk concentration c gm ions/l is greater than n, the number of fixed charges/cm² of surface.

~~~⊖ Pepsin ⊕ Cation

TABLE 5

Cation	k_1	k_2	N_1 Groups/cm²	$-\Delta G$ kcal/mol
Calculated from Eq. (7)				
K^+	9.55×10^6	7.4×10^2	2.69×10^{13}	6.1
Ba^{2+}	3.03×10^7	3.3×10^3	9.53×10^{12}	6.97
UO_2^{2+}	8.29×10^7	7.71×10^3	7.82×10^{12}	7.46
Al^{3+}	1.15×10^8	5.22×10^4	1.51×10^{12}	8.42
Th^{4+}	4.89×10^8	1.4×10^5	1.12×10^{12}	9.38
Calculated at zero point of charge from Eqs. (8) and (9)				
Al^{3+}	9.08×10^8	2.77×10^5	2.27×10^{12}	9.56
Th^{4+}	2.92×10^9	6.94×10^6	2.20×10^{12}	10.05
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The degrees of aggregation obtained in this manner agree well with the observed values as shown in Table 4.

Charge Density and Binding Parameters of the Cation Adsorption. *Charge Density.* Charge densities of the diffuse double layers at different zeta potentials have been calculated from the Eq. (6). A graph is plotted between σ_2/c and log molar concentration (Fig. 21) showing the changes in charge densities with the added amount of electrolytes. The charge is reversed at the higher concentration of Al(III) and Th(IV) ions. A model for the charge reversal in the presence of adsorbed pepsin has also been given (Fig. 22).

Binding Parameters. The binding parameters k_1 and k_2 have been calculated with the help of the Eq. (7). The constants are approximately determined by knowing the charge densities in Stern layer at two different concentrations of the electrolyte. From the values of k_1 and k_2 the constants N_1 and $-\Delta G$ have been evaluated and are tabulated in Table 5. In the case of $AlCl_3$ and $Th(NO_3)_4$ these constants have also been calculated by the treatment given in Eqs. (8) and (9) at zero point of charge. The constants are also of the same order as that of above calculated. The values are tabulated in Table 5. It is seen that with Al(III) and Th(IV) ions larger values of free energy of adsorption are obtained. This implies that charge reversal will take place at comparatively lower concentrations of these cations because larger the free energy of adsorption smaller is the amount of the counter ions to reach the critical value of charge reversal concentration.

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