

Electrolyte Flocculation of Haemoglobin-stabilized Toluene/Water Emulsion

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The stability of toluene in water emulsion stabilized by haemoglobin at pH 12 has been studied haemocytometrically and electrophoretically. The system is coagulated by various metal cations and the stability data are treated in the light of DLVO theory which shows the reversible flocculation in secondary minima. Adsorption constants, free energy of adsorption, number of binding sites available and the charge density in the Stern layer have also been calculated at zero point of charge.

MOORE and Krumbholtz¹ investigated the emulsifying efficiency of some proteins and found that acid and alkaline albumins were better than others. Reh binder and Trapeznikov² studied the viscoelastic properties of interfacial films stabilized by proteins. Similar studies were also made by Cumper and Alexander³. Charles Carr⁴ observed the binding of chloride ions to several proteins in the pH range 3-6. Rideal *et al.*⁵ have established that many proteins could be spread quantitatively at the air/water interface to form stable coherent insoluble films, whose average thickness corresponds closely to that of monolayer of fully extended polypeptide chain. The properties and structure of these films have been studied^{6,7} and similar studies have been made on protein monolayers spread at the benzene-water or petroleum ether/water interface⁸. The results of flocculation studies on haemoglobin-stabilized toluene/water emulsion are presented in this paper.

Materials and Methods

The emulsifying agent haemoglobin was a BDH product. The oil phase toluene and all the flocculating salts (nitrates of potassium, lead, barium, uranyl, lanthanum, chromium, thorium, and zirconium) were AR (BDH) chemicals. Doubly distilled water was used.

Emulsions were prepared by dispersing 5% toluene in aqueous solution of haemoglobin (0.1%) at pH 12 and ionic strength 0.01 maintained by adding KOH. The mixture was hand shaken for about 10 min and then passed through a stainless steel homogenizer to give emulsion droplets of average size (one micron radius).

The flocculation of emulsion particles by different electrolytes at different concentrations was observed by counting the number of oil drops haemocytometrically at different time intervals using an improved double Neubauer model. Counts were taken in 16 squares of the graticule fitted in the eyepiece (each square corresponded to a volume of

9×10^{-8} ml) with a hand tally counter (Eima, Tokyo) under an Olympus microscope (using 15×40 times magnifications).

Theoretical

The zeta potential was determined by micro-electrophoresis using a flat type cell⁹.

The total potential energies of the system were calculated by summing up the attraction and repulsion energies. The former was calculated using the Hamaker¹⁰ equation with the correction due to Schenkel and Kitchener¹¹. The repulsion energy was calculated using the equation due to Derjaguin and Kussakov¹². During these calculations the value of Hamaker constant was taken 1.0×10^{-13} erg which is very close to the value for pure toluene droplets dispersed in water¹³.

At the isoelectric point the adsorption constants are given by Eqs. (1 and 2)¹⁴

$$\frac{1}{C} = \frac{4\pi ev N_1 K_2}{\kappa \epsilon \Psi_{0\delta}} - K_2 \quad \dots(1)$$

$$\left\{ \frac{d\Psi_{\delta}}{d \ln C} \right\}_{\Psi_{0\delta}=0} = \left(\frac{\epsilon \Psi_{\delta} \kappa}{4\pi ev N_1} - 1 \right) \Psi_{0\delta} \quad \dots(2)$$

where C is the concentration of the flocculating electrolyte at zero point of charge and v is the valency of the counter ions, N_1 is the number of binding sites available per cm^2 , $(d\Psi_{\delta}/d \ln C)_{\Psi_{0\delta}=0}$ is the slope of the log C -zeta potential curves (assuming $\Psi_{\delta} = \xi_0$).

The adsorption constants K_1 and K_2 can be calculated from Eqs. (3 and 4)^{15,16}.

$$K_1 = N_1 K_2 ev \quad \dots(3)$$

and

$$K_2 = \frac{\exp(-\Delta G/kT)}{55.6} \quad \dots(4)$$

The charge density σ_s of the Stern layer has been calculated can also be from Eq. (5)

$$\sigma_s = \frac{K_1 C}{1 + K_2 C} \quad \dots(5)$$

ΔG , the electrochemical free energy of adsorption per molecule is given by Eq. (6)¹⁷

$$-\Delta G = kT (\ln K_2 + \ln 55.6) \dots(6)$$

Results and Discussion

Zeta potential — The fresh diluted emulsion has zeta potential -92.40 mV, which decreased gradually with increasing [flocculating salts]. Linear fall in zeta potential was observed for K^+ and Ba^{2+} ions. For rest the lowering in zeta potential was initially slow. After about an $7 \times 10^{-3} M$ electrolyte concentration zeta potential decreased rapidly, till the charge reversal took place. Further addition of flocculant caused rise in zeta potential which after certain concentration acquired constancy. Zeta potential values are plotted as a function of log [electrolyte] in Fig. 1.

Particle concentration — The emulsion initially contained 22.5×10^7 individual particles per ml. The particle number decreased with time and with increase in [electrolyte]. It is seen that the particles initially flocculated rapidly and after about 2 hr of emulsion preparation a very slow decrease in particle number is observed.

The half-life (the period at which the particle number becomes just half of initial) of emulsion decreases with [electrolyte] unless the zeta potential was very low. Emulsion with zeta potential less than about 10 mV flocculated immediately; microscopic studies at such concentration could not be made. Although no definite correlation between the zeta potential and half-life has been found but it is observed that at higher zeta potential values the half-lives were also higher.

Interaction energy and emulsion stability — In order to test the validity of the DLVO theory and

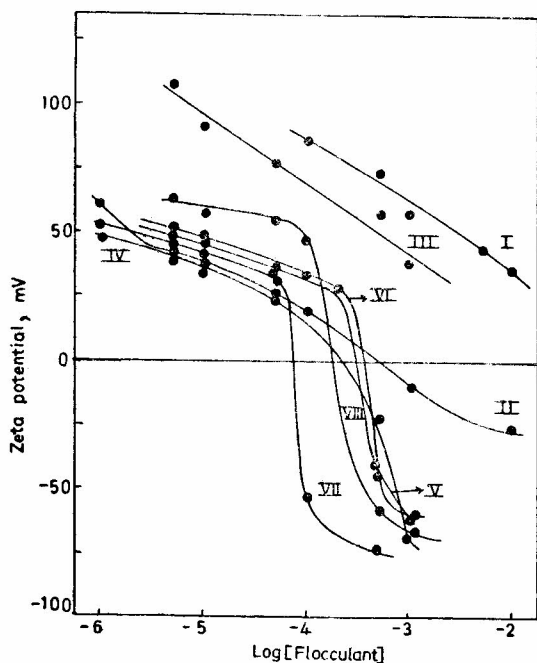


Fig. 1 — Variation in zeta potential with concentration in the presence of (I) KNO_3 , (II) $Pb(NO_3)_2$, (III) $Ba(NO_3)_2$, (IV) $UO_2(NO_3)_2$, (V) $La(NO_3)_3$, (VI) $Cr(NO_3)_3$, (VII) $Th(NO_3)_4$ and (VIII) $Zr(NO_3)_4$

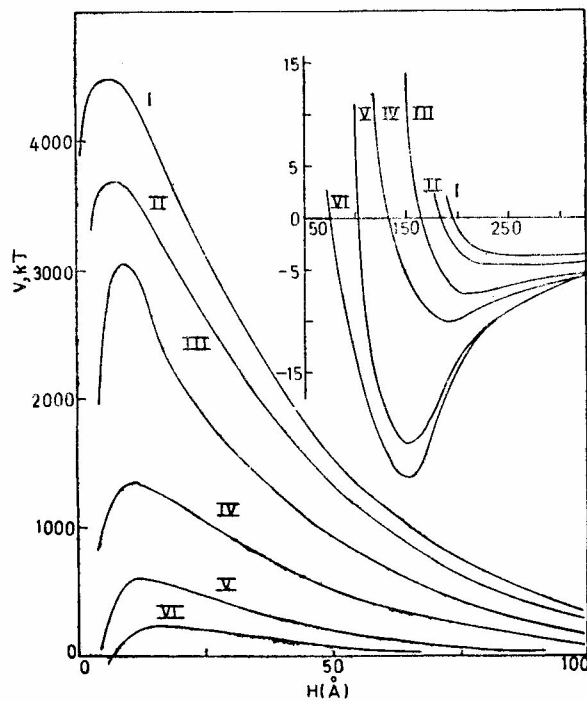


Fig. 2 — Variation in interaction energies with interparticle distance in presence of KNO_3 (I) 0.00, (II) 1×10^{-4} , (III) 5×10^{-4} , (IV) 1×10^{-3} , (V) 5×10^{-3} and (VI) 1×10^{-2} moles per litre

to study the nature of flocculation, the interaction energies V/kT were calculated and plotted as a function of interparticle distance. The curves are shown in Fig. 2 for KNO_3 . Similar trend in energy curves was observed for other electrolytes. The curves show the presence of very high potential energy barriers between the emulsified oil droplets which is caused by the large size of the droplets and high zeta values. These barriers are responsible for the high degree of stability of the emulsions.

Since the emulsions are flocculated at appreciable rates, the flocculation has thus been accounted to occur in the secondary minimum (i.e. a region of negative values of the potential energy at fairly large distance between the particles) of the potential energy of interaction. Such reversible flocculation which does not result in the immediate coalescence could be easily observed during the experiments. Enlarge portions of energy diagrams reveal 6-15 kT deep secondary minima at an interparticle distance of 150-200 Å. The enlarged secondary minima portions have also been presented in Fig. 2. The necessary numerical data are presented in Table 1.

Charge density and binding parameters — From the gradients of the curves at isoelectric points the different binding parameters, e.g. N_1 , the number of binding sites available, K_1 and K_2 , the adsorption constants $-\Delta G$, the free energy of adsorption, area etc. have been calculated and are presented in Table 2. The adsorbabilities of the counter ions at the surface of the emulsion particles are confirmed by the large values of K_2 , which show that higher the valency of the counter ions greater will be the

TABLE 1 — ZETA-POTENTIAL, INTERACTION ENERGY DATA OF THE EMULSION FLOCCULATED BY ELECTROLYTES

[Flocculant] <i>M</i>	$\times 10^{-6}$ (cm^{-1})	Mobility $\mu/\text{sec}/\text{volt}/\text{cm}$	Zeta- potential (mV)	Half life (min)	V_{max} (kT)	Distance at $V = 0$ (\AA)	Depth of secondary minima (kT)
KNO₃							
0.00	3.243	7.18	-92.40	—	4486	185-190	3.5
1×10^{-4}	3.261	6.62	-85.17	65	3660	180-185	4.5
5×10^{-4}	3.326	5.70	-73.35	50	3023	160-165	7.5
1×10^{-3}	3.403	4.44	-57.18	45	1347	130-135	10.0
5×10^{-3}	3.975	3.45	-44.42	40	580	105-110	22.0
1×10^{-2}	4.590	2.79	-35.93	—	212	75-80	25.0
UO₂(NO₃)₂							
1×10^{-6}	3.245	3.77	-48.5	—	850	145-150	6.0
5×10^{-6}	3.245	3.11	-40.00	—	466	125-130	12.0
1×10^{-5}	3.249	2.71	-34.88	180	296	115-120	14.0
5×10^{-5}	3.268	2.32	-29.94	100	80	95-100	22.0
5×10^{-4}	3.479	1.62	+20.92	55	—	55-60	24.5
1×10^{-3}	3.700	5.3	+69.0	42	2130	105-110	16.0
Ba(NO₃)₂							
5×10^{-6}	3.245	8.4	-108.45	—	6583	—	—
1×10^{-5}	3.249	7.05	-90.76	180	4289	193-195	6.0
5×10^{-5}	3.268	6.06	-77.99	105	2945	175-180	9.5
5×10^{-4}	3.294	4.49	-57.77	56	1368	130-135	12.5
1×10^{-3}	3.700	2.99	-38.55	43	367	110-115	23.0
Pb(NO₃)₂							
1×10^{-6}	3.245	4.88	-62.88	—	1764	170-175	9.5
5×10^{-6}	3.245	3.27	-42.10	—	543	125-130	11.0
1×10^{-5}	3.249	2.94	-37.82	189	390	105-110	13.0
5×10^{-5}	3.268	2.09	-26.98	135	100	100-105	22.0
1×10^{-4}	3.294	1.57	-20.23	90	—	60-65	24.0
1×10^{-3}	3.700	0.82	+10.52	46	—	—	26.5
La(NO₃)₃							
5×10^{-6}	3.249	3.80	-48.88	185	865	145-150	11.0
1×10^{-5}	3.255	3.61	-46.5	130	735	135-140	13.0
5×10^{-5}	3.294	2.71	-34.86	100	290	125-130	17.5
2×10^{-4}	3.433	2.01	-25.88	—	60	95-100	22.0
5×10^{-4}	3.700	3.15	+40.63	50	434	115-120	20.0
1×10^{-3}	4.104	4.74	+61.07	—	1494	120-125	19.0
Cr(NO₃)₃							
5×10^{-6}	3.249	4.02	-51.7	120	1026	130-135	10.5
1×10^{-5}	3.255	3.84	-49.52	100	897	125-130	11.5
5×10^{-5}	3.294	2.84	-36.58	65	341	115-120	17.5
1×10^{-4}	3.342	2.60	-33.51	50	241	110-115	19.5
2×10^{-4}	3.433	2.19	-28.22	—	91	100-105	23.0
5×10^{-4}	3.700	3.45	+44.47	45	565	105-110	21.5
1×10^{-3}	4.104	4.78	+61.58	—	1452	100-105	23.0
Th(NO₃)₄							
1×10^{-6}	3.245	4.17	-53.63	—	1143	160-165	8.0
5×10^{-6}	3.251	3.57	-46.00	140	718	140-145	9.0
1×10^{-5}	3.261	3.13	-40.39	100	482	115-120	10.0
5×10^{-5}	3.325	2.53	-32.59	80	230	100-105	13.0
1×10^{-4}	3.404	4.13	+53.19	55	1104	130-135	12.0
5×10^{-4}	3.975	5.73	+73.75	46	2505	140-145	11.0
Zr(NO₃)₄							
5×10^{-6}	3.251	4.93	-63.44	130	1800	155-160	8.0
1×10^{-5}	3.261	4.50	-57.91	105	1209	145-150	9.0
5×10^{-5}	3.325	4.21	-54.23	75	1172	135-140	10.5
1×10^{-4}	3.404	3.77	-48.61	50	832	120-125	13.0
5×10^{-4}	3.975	4.54	+58.41	45	1344	110-115	18.5
1×10^{-3}	4.590	5.27	+67.85	—	1872	105-110	22.0

TABLE 2 — BINDING PARAMETERS AT ZERO POINT OF CHARGE

Flocculating ions	[Flocculant] <i>M</i>	$\alpha \times 10^{-6}$ cm^{-1}	$K_1 \times 10^{-6}$	K_2	K_1/K_2	ΔG kcal/mole	σ_S coulombs/mole	$N_1 \times 10^{-12}$ groups/cm ²
Pb ²⁺	5.00×10^{-4}	3.479	16.13	3.64×10^3	4.43×10^4	5.71	6.88×10^3	46.17
UO ₂ ²⁺	2.24×10^{-4}	3.352	36.79	1.13×10^3	3.25×10^4	6.36	6.57×10^3	33.91
Cr ³⁺	3.55×10^{-4}	3.573	45.87	3.70×10^3	12.39×10^3	7.04	7.03×10^3	8.61
La ³⁺	3.16×10^{-4}	3.538	68.70	6.72×10^3	10.22×10^3	7.38	6.95×10^3	7.10
Zr ⁴⁺	1.78×10^{-4}	3.513	108.41	1.60×10^4	6.77×10^3	7.88	6.01×10^3	4.88
Th ⁴⁺	7.50×10^{-5}	3.360	274.94	2.83×10^4	9.71×10^3	8.21	6.60×10^3	5.06

adsorption of number of binding sites. This has also been confirmed by free energy data; smaller the free energy of adsorption the greater is the number of counter ions reaching the interface.

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