Influence of pH, Ionic Strength and Human Gamma Globulin Concentration on Stability of Polystyrene Latex Particle Suspensions.* (29028)

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Suspensions of polystyrene latex particles (LP) coated with human gamma globulin (HGG) have found widespread use in agglutination techniques for detection of the rheumatoid factors (RF) and other substances which react with human gamma globulin(1). Uncoated LP may also be agglutinated by RF containing sera(2). Other studies have employed uncoated LP to demonstrate agglutinating activity in dextran solution eluates prepared from erythrocytes from rheumatoid arthritis(3,4) and normal (4) blood.

Interpretation of LP agglutination by various substances is complicated by the fact that LP suspensions behave in some respects as do typical lyophobic colloids. The present report is therefore concerned with an investigation of the stability of LP suspensions as a function of pH, ionic strength and HGG concentration.

Methods and results. Latex particles used were Dow #568, 0.22 μ diameter, 49.5% solids, diluted in distilled water to 0.5 mg solids/ml. Various concentrations of HGG (Squibb, Human FII #1766)† in 0.7 ml 0.15 M NaCl were mixed with 0.3 ml 3 M NaCl and 2.0 ml of different pH buffers (pH 3.0 citrate; pH 4.0 to 5.6 acetate; pH 6.2 phosphate; pH 6.9 to 9.0, tris (hydroxymethyl) amino methane; $\gamma/2 = 0.03$). To this were added 2.0 ml of the above diluted LP suspension. The final mixture which contained 1.0 mg LP solids in 5.0 ml at $\gamma/2 = 0.21$ was incubated for 1 hour at 56°C, then maintained for 24 hours at 4°C, after which it was centrifuged (10 min, 800 $\times$ g) and examined visually for precipitation. The results are summarized in Table I. From pH 4.0 to pH 10.0, there was a minimum HGG concentration at which LP were unstable. Thus, at HGG concentrations of approximately 4 μg/mg LP, the particles precipitated spontaneously even though no specific agglutinator was present. At pH 4.0 and below, LP were unstable in the absence of HGG as well as in the presence of small amounts.

With increasing HGG concentration, a protective action was observed which was strongly pH dependent. In pH ranges below 4 and above 7.5, comparatively small amount of added HGG (<100 μg/mg LP) served to stabilize the LP suspensions. However, between pH 5.0-7.5 large amounts of HGG were required to stabilize the system. These data indicated that the protective action of HGG is minimal in the pH range 5.6-6.2. Extrapolation of Albertys' data(5) on the mean isoelectric point (pI) of HGG yields a value of 6.0 at $\gamma/2 = 0.21$. Thus it appears that the colloidal protective action of HGG is at a minimum at its own pI.

Data in Table II show that the protective action of HGG on LP stability is influenced

TABLE I. Influence of pH and HGG Concentration on Stability of Polystyrene Latex Particles ($\gamma/2 = 0.21$).

pH	HGG concentration range in which LP spontaneously precipitate (μg/mg LP/5 ml)
1.0	0— 33
2.0	0— 33
3.0	0— 65
4.0	1.2— 98
4.5	3.5— 483
5.0	3.5— 980
5.6	2.3—32,250
6.2	4.7—32,250
6.9	3.5—16,120
7.5	3.5— 8,062
8.0	2.3— 98
9.0	4.7— 65
10.0	4.7— 65

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TABLE II. Influence of Ionic Strength on Maximum Concentration of HGG at Which Spontaneous Precipitation of Latex Particles Occurs (pH 6.90).

Ionic strength	HGG added (μg/mg LP/5 ml)
.01	114,000
.07	64,500
.13	32,400
.19	16,100
.25	15,400
.31	15,400
.37	8,100
.43	9,400
.49	9,600
.55	11,200

by the ionic strength of the system. The experiment was done at pH 6.9 as previously described, but with varied ionic strength produced with different concentrations of NaCl. At ionic strengths below 0.19 the protective action of HGG was sharply reduced and larger quantities of HGG were required to stabilize the LP suspensions. From $\gamma/2 =$ 0.19-0.55, the protective action of HGG was less sensitive to ionic strength and some increase in protective ability was noted at the higher ionic strengths.

In the above experiments, stability of LP was studied in the presence of excess HGG. To assess stability of HGG coated LP in the absence of excess HGG, the original stock LP suspension was diluted 10-fold and subjected to prolonged dialysis against running tap water and later against several changes of distilled water. An amount of LP suspension containing 1.0 g solids was mixed with a 0.15 M NaCl solution containing 0.5 g HGG to a final volume of 100 ml ($\gamma/2 = 0.015$). This mixture was allowed to stand for 1 hour after which the mixture was centrifuged (20 min, 13,300 $\times$ g) and the supernatant discarded. The pellets of HGG coated LP were washed in a total of 20 ml of 0.015 M NaCl and recentrifuged. The supernatants were discarded and the pellets suspended in 3.0 l of distilled H_2O. Mixtures were prepared consisting of 4 ml coated LP suspension, 4 ml of the various buffers, and 4 ml of varying NaCl concentrations. The above mixtures, containing 1 mg LP solids/ml, were incubated for 1 hour at 56°C, refrigerated at 4°C for 24 hours, centrifuged (10 min, 800 $\times$ g) and then examined for precipitation. Stability of coated LP was dependent on both pH and ionic strength (Table III). In general, coated LP were unstable at pH 6-8. With increasing ionic strength the instability range was shifted to a more acid pH range. Finally at very high NaCl concentrations ($\gamma/2 > 1.0$), instability was observed throughout the whole pH range studied.

Discussion. The upper limit for the diameter of colloidal particles is generally given as 0.1 μ(6). The diameter of the LP used in this study was 0.22 μ and the diameter of LP usually employed in agglutination tech-

TABLE III. Influence of pH and Ionic Strength on Spontaneous Precipitation of HGG Coated Latex Particles.

Ionic strength	pH										
	3.0	4.0	4.5	5.0	5.6	6.2	6.9	7.5	8.0	9.0	10.0
.01	–	–	–	–	–	3	3	3	–	–	–
.06	–	–	–	–	3	3	3	3	3	–	–
.11	–	–	–	–	3	3	3	3	2	–	–
.16	–	–	–	1	3	3	3	3	1	–	–
.21	–	–	–	1	2	3	3	3	–	–	–
.26	–	–	1	1	2	3	3	3	–	–	–
.31	–	–	1	2	2	3	3	3	–	–	–
.36	–	2	2	2	2	3	3	3	–	–	–
.41	–	2	2	2	–	3	3	1	–	–	–
.46	–	3	2	2	1	2	2	1	–	–	–
.51	–	3	2	2	2	2	2	1	–	–	–
.71	3	3	3	2	1	2	2	1	–	–	–
1.01	3	3	3	2	–	2	2	3	2	–	–
2.01	3	3	3	3	3	3	3	3	2	1	–
3.01	3	3	3	3	3	3	3	3	3	3	3

3. Complete precipitation.
2. Partial precipitation.
1. Trace of precipitation.
– No precipitation.

niques is 0.81 μ, both of which are above this upper limit.

Nevertheless, these LP suspensions may be regarded as colloidal in nature, at least with respect to their stability properties in the presence of a comparatively lyophillic protective colloid such as HGG. In the presence of HGG, LP exhibits the properties of lyophobic colloid. As small amounts of HGG are added, the LP are destabilized and rapid coagulation occurs. This effect has been ascribed to the lowering of the LP electrokinetic or "zeta" potential by the added protein(7). As more HGG is added, the particle surface is covered with protein and the coated particles assume some of the properties of the adsorbed protein. It has been estimated(8) that a monolayer of adsorbed HGG on 0.22 μ diameter LP consists of 163 and 178 μg HGG/mg LP at pH 3.98 and pH 9.01 respectively. Under such conditions (Table I) LP are stable. Stability is therefore associated with the addition of sufficient HGG to form an adsorbed monolayer on the LP. This observation holds only at pH values differing by at least $\pm$ 2 units from the protein pI. The protective ability of HGG decreases at pH values approaching the protein pI, due to the decreased net charge and decreased solubility of the protein.

The effect of ionic strength is more complex. The protective action of HGG is enhanced with increasing ionic strength (Table II), probably due to increased protein solubility. On the other hand, increased ionic strength also increased the pH range in which coated LP were unstable (Table III). In this case, the effect might be ascribed to the reduction in effective charge of the protein by the added electrolyte. This would decrease interparticle repulsion and favor instability.

The interpretation of agglutination of uncoated LP by various systems(3,4) is complicated by the fact that with conditions of low protein concentration, suspensions of LP may precipitate spontaneously even in the absence of specific agglutinating substances (1,9). Agglutination techniques for RF detection employing HGG coated LP are commonly performed at pH values above 8. At such pH values with the protective coating of HGG, LP are stable. Consequently, it can be expected that under these conditions agglutination occurs only as a result of the reaction between RF in solution and HGG adsorbed to the LP surface.

Summary. Polystyrene latex particle suspensions exhibit the properties of lyophobic colloids. Addition of small amounts of human gamma globulin serves to destabilize the LP whereas larger amounts exert a stabilizing protective action. The protective action of HGG is strongly pH dependent and is least at the protein isoelectric point. Use of uncoated latex particles for detection of rheumatoid factors may be complicated by the fact that such particles spontaneously precipitate in very dilute protein solutions.

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