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Influence of Electrolytes on Stability of 0.2 Micron Diameter Polystyrene Latex Particles.*† (29831)

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(Introduced by H. M. Zimmerman)
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Polystyrene latex particles 8000 Å in diameter were utilized in the original Latex Fixation Test(1). Since that time, modifications of the original procedure using latex particles from different sources and of different sizes(2) have resulted in considerable variability of titers from laboratories testing standard rheumatoid arthritis sera. This variation has drawn attention to the need for further standardization of the materials used in this test(3).

The polystyrene latex particles are prepared in various sizes from 1) a latex monomer, 2) a polymerizing catalyst or initiator, 3) water, and 4) an emulsifying agent. Potassium persulfate, potassium percarbonate, potassium perborate, potassium hydroxyperoxide or gamma rays from Co^{60} may serve as the initiator. An anionic emulsifier such as Triton X may be employed in preparation of these particles(4,5). The amount of emulsifier adsorbed on the particles varies with their size and so may affect their serological reactivity.

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The purpose of the investigation reported here was to study some physical properties of latex particles—namely, their stability, both uncoated and coated with human gamma globulin, in the presence of several electrolytes at various ionic strengths and pHs. On the basis of studies reported elsewhere(6,7) latex particles 2000 Å in diameter were used.

Materials. Lytron #615‡ polystyrene latex particles ranging in size between 1500 and 2500 Å in diameter were employed. As obtained from the manufacturer, the particles have the following characteristics: 1) negative charge, 2) a pH of 10.3 ± 2 in an aqueous suspension, 3) a specific gravity of 1.03 and 4) a viscosity of 20 to 40 centipoises at 25°C. They are unchanged by freezing and thawing(8).

The latex suspension was diluted with distilled water until 0.1 ml of the diluted suspension added to 10 ml of water matched the optical density of a standard barium sulfate suspension, as measured in a Coleman Jr. spectrophotometer at 650 mμ. The diluted latex suspension, which contained 30.6 mg of dry latex per ml of solution, was used in the preparation of uncoated latex particles and latex particles coated with gamma globulin. The standard barium sulfate suspension was prepared by adding 3 ml of

‡ Obtained through the courtesy of Monsanto Chemical Co., Springfield, Mass.

0.10 N barium chloride to 3 ml of ⅔ N sulfuric acid and heating the mixture at 56°C for one minute.

One hundred ml of the latex suspension was centrifuged for 15 minutes at 17,500 to 20,180 × *g*, and the sediment washed 3 times in distilled water and resuspended in 95 ml of water, so that the optical density again equalled that of the standard barium sulfate suspension.

Part of the latex suspension was dialyzed for 2 days against running tap water and for 2 days against 4 changes of distilled water. The resultant suspension was kept as the dialyzed, uncoated latex stock suspension. Lyophilized Squibb serum fraction II, lot #1766,§ was the human gamma globulin used for coating. This material was dissolved in 0.15 M sodium chloride to a concentration of 46.2 mg per ml. One part water, 2 parts of this globulin solution and 2 parts of the dialyzed, uncoated latex stock suspension were mixed, allowed to stand for 30 minutes at 24°C, and centrifuged for 15 minutes at 17,500 to 20,180 × *g*. The sediment was washed 3 times with distilled water to remove excess globulin, dialyzed in the same manner as the uncoated particles, and the volume adjusted so that the suspension in a hundred-fold dilution equalled that of the standard barium sulfate suspension.

The following buffer solutions, all at an ionic strength of 0.03, were prepared: 1) citrate buffer, pH 3.1, 2) acetate buffers, pH 4.5, 5.0, 5.6, 3) tris (hydroxymethyl)-aminomethane buffers, pH 6.9, 7.5, 8.0, 9.0, and 4) borate buffer, pH 10.0.

Methods. The stability of the dialyzed and undialyzed uncoated latex suspension between pH 3.1 and 10.0 was determined by mixing equal parts of buffer and a 1:100 dilution of the uncoated latex particle suspension in water. The mixtures were incubated overnight at 56°C, centrifuged at 1536 × *g*, and the optical density of the decanted supernatant determined at 650 mμ in a Coleman Jr. Spectrophotometer.

Stability was considered to be represented by a uniform colloidal suspension of latex particles with no visible agglutination or aggregation, and by a turbidity corresponding to that of the barium sulfate standard. Instability was indicated by visible agglutination and an optical density of zero. Partial instability was indicated by visible agglutination and an optical density between that of the standard and zero.

The effect of electrolytes on the uncoated and coated latex suspensions at pH 5.0 and 9.0 was determined in the following manner. Four rows of 10 tubes, each containing 2.5 ml of progressive 2-fold dilutions of the electrolyte under study were prepared. To 2 rows of tubes, 2.5 ml of pH 9.0 buffer was added. Five ml of a 1:100 dilution of the dialyzed, uncoated latex stock suspension were added to each tube in one row at pH 5.0 and each tube in one row at pH 9.0. The same procedure was done with the coated stock suspension. Controls of gamma globulin coated latex and uncoated latex particles in buffer without electrolytes were prepared. All tubes were incubated, centrifuged, and the supernatant read in the spectrophotometer at 650 mμ.

Results. The uncoated latex particles, dialyzed and undialyzed, were found to be stable between pH 3.1 and 10.0 in the absence of salt (Table I). Addition of 0.25 M sodium chloride caused the undialyzed latex particle suspension to be unstable between pH 3.1 and 6.9. One molar sodium chloride produced instability of the undialyzed latex suspension up to pH 8.0 and of the dialyzed particles up to pH 10.0.

Table II shows the stability of uncoated latex particles in the presence of various salts at pHs of 5.0 and 9.0. In general, trivalent cations (*e.g.*, Al^{+++}) at concentrations as low as 0.26×10^{-3} M caused instability of the uncoated latex. Divalent cations at higher concentrations caused instability while monovalent ions were least effective in reducing the stability of the latex suspension.

The effect of the various electrolytes on the stability of the coated latex particles showed a dependence on pH (Table III). At pH 5.0 the trivalent cation Al^{+++} had no effect upon the particles, and the divalent

§ Obtained through the generosity of American Red Cross and E. R. Squibb and Sons.

TABLE I. Ability of Dialysis, pH, and NaCl Concentrations to Decrease Stability of Uncoated Latex Particle Suspensions.

NaCl molarity	pH 3.1	4.0	4.5	5.0	5.6	6.9	7.5	8.0	9.0	10.0
A. Undialyzed Latex Particle Suspensions										
1.0	+	+	+	+	+	+	+	+	—	—
.25	+	+	+	+	—	—	—	—	—	—
.00	—	—	—	—	—	—	—	—	—	—
B. Dialyzed Latex Particle Suspensions										
1.0	+	+	+	+	+	+	+	+	+	+
.25	+	+	+	+	+	+	—	—	—	—
.00	—	—	—	—	—	—	—	—	—	—

"+" Decrease in stability. "±" Partial decrease in stability. "—" No decrease in stability.

TABLE II. Ability of Various Salts to Decrease Stability of Uncoated Latex Particle Suspensions.*

	Molarity × 10^{-3} 167	84	42	21	11	5.2	2.6	1.3	.52	.26
A. pH 5.0										
$AlCl_3$†	+	+	+	+	+	+	+	+	—	+
$CaCl_2$	+	+	+	+	±	—	—	—	—	—
$MgCl_2$	+	+	+	+	±	—	—	—	—	—
$BaCl_2$	+	+	+	+	±	—	—	—	—	—
Na_2HPO_4	+	±	—	—	—	—	—	—	—	—
Na_2SO_4	+	+	—	—	—	—	—	—	—	—
B. pH 9.0										
$AlCl_3$	+	+	+	+	+	+	±	±	±	—
$CaCl_2$	+	+	+	+	+	±	—	—	—	—
$MgCl_2$	+	+	+	±	—	—	—	—	—	—
$BaCl_2$	+	+	+	+	—	—	—	—	—	—
Na_2HPO_4	—	—	—	—	—	—	—	—	—	—
Na_2SO_4	—	—	—	—	—	—	—	—	—	—

* Uncoated latex particles were found to be stable at all concentrations tested of NaCl, NaH_2PO_4, Na_2 citrate, $Na_2C_2O_4$ and Na_3PO_4.

† Uncoated latex particles were stable from 1.0×10^{-3} to 0.4×10^{-3} M $AlCl_3$ and thereafter unstable to 1.0×10^{-5} M $AlCl_3$ and stable from 5×10^{-6} and thereafter.

"+" Decrease in stability. "±" Partial decrease in stability. "—" No decrease in stability.

cations were only weakly effective in producing instability. On the other hand, the divalent anions were all capable of causing instability of the coated latex suspension.

At pH 9.0 the salt effects were reversed. The coated latex particle suspension was stable in the presence of all the divalent anions tested, but unstable in the presence of polyvalent cations.

Discussion. It has been shown that the stability of a latex particle suspension depends upon the electrostatic forces of repulsion, which predominate over those of attraction. These repulsive forces are believed to be caused by the emulsifier ions which form an electric double layer on the surface of the hydrophobic polymer particles. This electric double layer is generally negatively charged. Repulsion of the particles will occur until the distance between them is reduced and the London-Van der Waals forces of attraction become predominant. The presence of sufficient emulsifier ions results in a strong repulsive force producing a stable suspension(5,6,9).

Negatively charged particles have their highest charge at alkaline pH, thereby resulting in increased stability of the suspension (Table I). The decreased stability of dialyzed latex particles as compared to un-

TABLE III. Ability of Various Salts to Decrease Stability of Human Gamma Globulin Coated Latex Particle Suspensions.

	Molarity × 10^{-3}									
	167	84	42	21	11	5.2	2.6	1.3	.52	.26
A. pH 5.0										
$AlCl_3$	—	—	—	—	—	—	—	—	—	—
$CaCl_2$	±	±	±	—	—	—	—	—	—	—
$MgCl_2$	±	±	—	—	—	—	—	—	—	—
Na_2 citrate	+	+	+	+	+	—	—	—	—	—
Na_2SO_4	+	+	+	±	±	±	—	—	—	—
K_2HPO_4	N.D.	+	+	+	+	+	—	—	—	—
$Na_2C_2O_4$	N.D.	N.D.	+	+	±	±	—	—	—	—
B. pH 9.0										
$AlCl_3$	—	—	+	+	+	+	±	±	±	—
$CaCl_2$	+	+	+	+	+	+	±	—	—	—
$MgCl_2$	±	±	±	±	±	—	—	—	—	—
$Na_2C_2O_4$	N.D.	N.D.	—	—	—	—	—	—	—	—
Na_2 citrate	—	—	—	—	—	—	—	—	—	—
Na_2SO_4	—	—	—	—	—	—	—	—	—	—
K_2HPO_4	N.D.	—	—	—	—	—	—	—	—	—

N.D. = Not done.
"+" Decrease in stability. "±" Partial decrease in stability. "—" No decrease in stability.

dialyzed particles may be explained by the fact that dialysis removes part of the emulsifier and therefore part of the charge, resulting in a somewhat less stable suspension in the presence of sodium chloride.

Addition to the particle suspension of an electrolyte such as sodium chloride causes a greater potential gradient and results in a decreased zeta potential of the layer that actually adheres to the particle, thus decreasing the stability (Tables I and II). On the other hand, the lower the salt concentration, the more extended the electrical double layer and the greater the zeta potential with a resultant increase in stability of the suspension.

Schulze(10,11) and Hardy(12) have shown that the nature of ions added to a hydrophobic colloid influences its stability. The "counterions," those charged oppositely to the particles, exert the greatest influence. Tables IA and IIB demonstrate that fewer trivalent counterions are needed to cause instability of the colloid than divalent counterions, and fewer divalent than monovalent counterions. At pH 9.0, even one molar sodium chloride will not decrease stability, whereas 5.2 millimolar calcium chloride will, and only 0.52 millimolar aluminum chloride will. The relative inefficacy of anions in reducing stability is seen in Table II.

A reversal of charge produced by the adsorption to the latex particles of positively charged colloidal oxides or hydroxides of aluminum(13,14) is demonstrated in Table IIA. Between 0.01 and 0.3 millimolar aluminum chloride the particles apparently changed from being negatively charged to being uncharged, causing instability of the suspension. Between 0.4 and 1.0 millimolar aluminum chloride, the now positively charged articles had just sufficient charge to be stable. At higher concentrations, the feeble positive charge again diminished and could no longer stabilize the system(15).

When the lyophobic latex particles are coated with human gamma globulin, a lyophilic colloid, the particles acquire the stability characteristics of the colloid(13). The resultant suspension is stable when the particles are completely, or almost completely, coated. The suspension then reacts as if it were a colloidal suspension of gamma globulin particles. However, when the particles are incompletely coated, the suspension may become unstable because of the neutralization of small amounts of an oppositely charged lyophilic colloid(13). It has been demonstrated that gamma globulin concentrations of less than 5 μg per mg of latex particles will reduce the stability of latex particle suspensions(16).

Table III illustrates the amphoteric nature

of the coated latex particles suspension. At pH 5.0, the particles are positively charged; at pH 9.0, they are negatively charged. In accordance with the previously mentioned laws of Schulze and Hardy, at pH 5.0, divalent anions cause instability, while at pH 9.0, polyvalent cations caused instability. The phenomenon of reversal of charge with aluminum chloride is again illustrated in Table IIIB. The higher initial negative charge of the gamma globulin coated particles at pH 9.0 as compared to the uncoated particles at pH 5.0 (Table IIA) can explain the fact that reversal of charge took place at a much higher concentration of aluminum chloride with the coated latex particles.

Generally, agglutination procedures utilizing human gamma globulin are performed above pH 8.0. From the data presented here, it would be expected that serum electrolytes in their usual concentration would not appreciably affect the agglutination reaction. However, in other fluids or when particles are coated with protein material, the presence of divalent or trivalent cations may reduce the stability of the latex particle suspension with consequent production of a false positive test.

Summary. The stability of polystyrene latex particles, 2000 Å in diameter, in suspension, both uncoated and coated with human gamma globulin, was studied as a function of pH, electrolyte concentration and valence. Uncoated latex particle suspensions became unstable in the presence of polyvalent cations but not anions. When particles were coated with human gamma globulin, they acquired the characteristics of the protein coating, including its amphoteric properties. At pH 5.0 (net charge positive) the presence of polyvalent anions resulted in instability of the coated particles, while at pH 9.0 (net charge negative) the coated particles became unstable in the presence of polyvalent cations.

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