Rapid Papers

Density-Gradient Sedimentation in Silica Sols

ANOMALOUS SHIFTS IN THE BANDING DENSITIES OF POLYSTYRENE 'LATEX' BEADS

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When polystyrene 'latex' beads are centrifuged to equilibrium in gradients of Nalcoag 1030, Nalcoag 1034A and Ludox 130M, which are commercial formulations of colloidal silica, the beads band at densities that may be markedly lower or higher than the bulk density. Addition to the gradient of small amounts of certain polymers restores the banding densities towards the expected value. These findings closely mimic previously observed 'density shifts' of biological particles. A possible model for this anomalous behaviour is discussed.

Silica sols, such as 'Ludox', were first used as a density-gradient material by Mateyko & Kopac (1963) for the 'isopyknotic cushioning' of cells from animal tissue cultures. Subsequently Pertoft and co-workers (see Pertoft & Laurent, 1969) established the general applicability of silica sols in density-gradient centrifugation in a systematic investigation of the conditions for separating several viruses and a wide variety of membrane-bound particles. More recently the range of particle separations possible with silica sols has increased substantially.

In a study of the sedimentation of zooplankton in gradients of Ludox AM, St. Onge & Price (1975) observed a curious shift in the banding densities of fish larvae and of the arrow-worm *Sagitta* when low concentrations of certain substances, notably anionic polymers, were added to the gradients. We observed similar 'density shifts' with chloroplasts and thylakoid membranes when polyethylene glycol was added to gradients of Ludox AM (Morgenthaler *et al.*, 1975). In both the zooplankton and the chloroplast studies, the density shifts were essential to successful separations.

We think it most remarkable that the banding density of a particle should be strongly affected by subtle changes in the composition of a gradient and, further, that the extent and even the direction of density shift should be so specifically dependent on the nature of the particle. In order to place density shifts on a more rational basis, we sought a system simpler than the organelles or organisms found in Nature. We found that suspensions of very small beads of polystyrene 'latex' show anomalous banding

densities in gradients of silica sols and that their banding densities are also modified by density-shift reagents.

Experimental

Several silica sols were employed: Nalcoag 1030, with a pH of the delivered sol of 10.2, is composed of silica bearing negative charges; Nalcoag 1034A (pH: 3.1) bears no net charge; and Ludox 130M (pH: 4.4) bears positive charges. The Nalcoags and Ludox 130M respectively were generously provided by Nalco Chemical Co., Chicago, IL 60601, U.S.A., and E. I. du Pont de Nemours, Industrial Chemicals Department, Wilmington, DE 19898, U.S.A. The pH of the sols was adjusted by titration with sulphonic acid-type ion-exchange resins in the H⁺ form or quarternary ammonium resins in the OH⁻ form and filtering off the resin. Although these treatments also removed counterions, we found that Nalcoag 1030 was stable at least to pH 6. Nalcoag 1034A was stable over a range of at least pH 2 to pH 5, and Ludox 130M was stable down to pH 3. Polystyrene latex beads, 0.316 μ m and 1.011 μ m in diameter, and polystyrene-divinylbenzene beads, 5.7 μ m in diameter, were used. The beads also differ in surface properties. They are not simply spheres of the polymer or the co-polymer; rather their surfaces are modified with sulphuric acid and sulphonic acid residues, which confer stability, in the case of the two smaller-size beads. The 5.7 μ m beads are stabilized with a protective colloid, which surrounds each polystyrene bead. Polyethylene glycols 400, 1000, 6000 and 20M were obtained as Carbowax from Union Carbide Chemicals and Plastics Division, Charleston, WV 25303, U.S.A.; dextran sulphate and Ficoll 70 were from Pharmacia, Uppsala, Sweden; Tween-20 and

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trimetaphosphate were from Sigma Chemical Co., St. Louis, MO 63178, U.S.A.; polyvinyl alcohol was from Matheson, Elk Grove Village, IL 60007, U.S.A.; polyvinyl sulphate was from Eastman Kodak, Rochester, NY 14650, U.S.A.

Gradients were 12.5 ml and linear with volume. Small quantities (approx. 0.2 mg) of latex were introduced at the top or the bottom of the gradients and normally centrifuged for 45 min at 7000 rev./min in an SB283 rotor, corresponding to 5800g at $r_{av.} = 10.5$ cm (Damon/IEC, Needham Heights, MA 02194, U.S.A.). Each gradient was monitored by puncturing the tube from the bottom in a no. 3851 tube fractionator (Damon/IEC), the stream was read at 700 nm in a Gilford spectrophotometer, the absorbancy profile was recorded and 0.5 ml fractions were collected. The densities of individual fractions were determined by a method described by Morgenthaler *et al.* (1975) and based on the methods of Linderström-Lang (1937) and Oster (1965).

Results

The density of polystyrene latex particles has been reported to be 1.055 ± 0.001 g/ml (Kahler & Lloyd, 1951). The density of the styrene-divinylbenzene particles can be expected to be somewhat more dense, from the admixture of 5% (w/v) of divinylbenzene of density 1.11 g/ml. In gradients of sucrose and NaCl the latex particles sediment to narrow bands at densities close to 1.05 g/ml. In Nalcoag, however, the banding densities of the two smaller beads are decreased to 1.034 and 1.036 g/ml, values that are significantly different from the bulk density. Indeed, the repeatability of individual sets of values is about 0.002 g/ml, and the individual bands show a dispersion of 0.01 g/ml. In Ludox 130M, on the other hand, we observed a density of 1.056 g/ml for the beads $1.011 \mu\text{m}$ in diameter. The largest beads show uniformly almost the same density of 1.06 g/ml in the several kinds of silica, as well as in sucrose or NaCl gradients. In critical cases the banding densities were confirmed by both sedimentation and flotation, thus ensuring that the reported densities correspond to secular equilibrium.

Thus we see that solid osmotically insensitive particles can show anomalous banding densities in gradients of silica sols.

To determine whether this phenomenon is in any way similar to the density shifts observed with biological particles, we then tested the effects of a number of the density-shift reagents identified by St. Onge & Price (1975) on the banding densities of latex beads, and we also examined the effects of pH over the compatible ranges of the three silica sols (Fig. 1). What we see is that substances as different chemically as dextran sulphate, Tween-20, polyvinyl alcohol and polyethylene glycol rather uniformly increase the

Fig. 1. Effects of density-shift reagents on the banding densities of polystyrene latex beads $1.011 \mu\text{m}$ in diameter at various pH values

Experimental details are given in the text. The stippled band indicates the banding density of the latex beads in gradients of sucrose and NaCl. Also shown are the banding densities in the presence of the following density-shift reagents: $\square$, Nalcoag 1034A; $\circ$, Nalcoag 1030; $\bullet$, Nalcoag+3% polyethylene glycol 6000; $\triangle$, Ludox 130M.

banding densities towards that of the bulk density, i.e. they cause a density shift from 1.034 to about 1.045 g/ml. Even with the uncharged silica sol, Nalcoag 1034A, trimetaphosphate shifts the density of the beads from 1.01 to 1.05 g/ml!

Discussion

Latex beads show anomalous banding densities and density shifts when sedimented in certain silica sols. Unlike biological particles, where we cannot be certain of their true densities, we can say that latex beads tend towards anomalously low densities when centrifuged in sols containing negatively charged particles, and that density-shift reagents move the banding densities towards their true or bulk densities.

Can we then argue by analogy that biological particles behave like latex beads? Qualitatively, at least, fish larvae and intact spinach chloroplasts respond to some of the density-shift reagents as do latex particles, whereas the thylakoid membranes of chloroplasts respond by decreasing their banding densities (St. Onge & Price, 1975; Morgenthaler *et al.*, 1975).

Our finding is probably not the first indication of anomalous banding densities of latex beads in silica sols. Pertoft (1966) described how a series of different sizes of latex beads ranging in diameter from 0.088 to $1.305 \mu\text{m}$ could be resolved in gradients of Ludox HS containing 5% dextran, although these same beads banded together in gradients of sucrose. Pertoft

(1966) did not compare the absolute densities of the particles in the two kinds of gradients, but his data show that the apparent densities of the latex beads in silica decrease progressively with decreasing size of the particles. Our data, which are restricted to a limited range of particle sizes, are inconclusive on this point.

It is difficult to construct a consistent model that can account for the twin phenomena of anomalous banding densities and density shifts. In a different connexion Pertoft & Laurent (1969) proposed a hypothesis of silica exclusion (Fig. 2), in which it is supposed that colloidal silica is somehow excluded from the regions of the solution near the particles. Repulsive forces could conceivably arise from like charges on the silica and the particles in question. The silica-exclusion space would then be occupied exclusively by solvent and the small molecules dissolved in the solvent. Since the silica-free solvent would be less dense than solvent-cum-silica, particles would then behave as if they were larger and less dense.

One can speculate further that density-shift reagents act by coating the silica surfaces and thus insulate them from the repulsive forces of the particles (Fig. 2*b*) specifically by decreasing the dielectric strength. It is well known (Pertoft, 1969; Rupprecht & Liebl, 1974) that polymers, such as polyethylene glycol, adsorb on silica surfaces. We can imagine a less likely alternative where density-shift reagents might accumulate on the surface of the sedimenting particle, thus increasing its density.

If the silica particles and the latex particles being separated bear opposite charges, an adsorption of the

heavy SiO_2 particles on the surface of the latex beads would cause them to band at a density higher than their bulk density (Fig. 2*c*).

Let us now examine the silica-exclusion hypothesis quantitatively. As shown in Fig. 2, the significant quantities are the radius of the particle r_p , the radius from the centre of the particle to the edge of the hypothetical exclusion volume r_a , the true density of the particle ρ_p and the apparent or banding density of the particle ρ_a . The relations among these can be expressed as:

$$\frac{r_a}{r_p} = 3 \sqrt{\frac{\rho_p - 1}{\rho_a - 1}}$$

The exclusion radius $r_e = r_a - r_p$ should be dependent on the nature of the surfaces of silica and the particles in question, and should therefore be independent of the size of the particle. An immediate deduction from the model is that the apparent density will become smaller the smaller the particle, i.e. as the ratio of exclusion volume to particle volume increases. This indeed seems to be what Pertoft (1966) observed.

However, when we insert experimental values into the equation, a serious problem arises. With particles of 1.011 μm diameter, a ρ_p of 1.05 and a ρ_a of 1.02 g/ml, the silica-exclusion radius is calculated to be 180 nm! It is extremely difficult to imagine electrostatic forces that could exclude silica particles over such enormous distances.

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Fig. 2. Possible models for the effect of density-shift reagents on the banding density of polystyrene latex beads

For explanation see the text.

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