A METHOD FOR SEPARATING ACCORDING TO MASS A MIXTURE OF MACROMOLECULES OR SMALL PARTICLES SUSPENDED IN A FLUID, II. EXPERIMENTS IN A GRAVITATIONAL FIELD

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We have described a method for separating according to mass a mixture of macromolecules or small particles.[1] The particles are suspended in a fluid and exposed to a uniform acceleration. The separation occurs as a consequence of the motion of the fluid. If the particles are heavy, the method works in the earth's gravitational field; if they are light, centrifugal fields are required. We will describe here experiments designed to test the method using heavy particles and a gravitational field. A third paper will discuss experiments done in a centrifugal field.[2]

The apparatus used in the gravity experiments, a long rectangular trough, is shown in schematic side view in Figure 1. A glass plate on the bottom of the trough is covered with a thin layer of fluid. The particles to be separated are added as a spot or a narrow band near one end of the trough. The gravitational field concentrates the particles near the surface of the glass in exponential Boltzmann distributions. The height of a distribution depends on what we have called the effective mass of the particle, the mass of the particle less the mass of the fluid it displaces. The lighter the particle, the higher its distribution. The fluid is made to flow slowly down the length of the trough. In this laminar flow the velocity of the fluid increases with height. The higher the particle, the more rapidly it moves down the trough. Light particles are on the average higher in the fluid than heavy particles; therefore, they travel more rapidly. At any moment, however, the particles in a given distribution have various heights and therefore various velocities. Brownian motion of an individual particle causes it to sample all these heights and velocities. Consequently, the average velocity of one particle is nearly the same as that of another particle of the same kind, and particles of the same kind move as a group. The method is capable of high resolution only if enough time is allowed for this sampling to occur.

Materials and Methods.—Particles: We used "monodisperse" polystyrene latex spheres of diameter 0.796 μ (run no. LS-449-E) and 1.305 μ (run no. LS-464-E) obtained from the Dow Chemical Company Bioproducts Department through the courtesy of L. S. Lippie. The diameters had been measured at Dow in an electron microscope by the method of Bradford and Vanderhoff.[3] The particles came in an aqueous suspension (10% solids) containing an alkyl benzenesulfonate emulsifier. We diluted the 1.3-μ suspension about 1 : 20 and the 0.8-μ suspension about 1 : 6 with the fluid to be used in the experiment and allowed the mixtures to settle at least six hours in columns about 1.5 cm high. The suspensions used in the experiments were withdrawn from the middle of these columns. We measured the density of this material by observing whether the particles would float or sink when centrifuged in sucrose solutions of graded concentrations, obtaining a value 1.051 ± 0.001 gm/cm^3 (25°C).

Fluid: Our preliminary experiments with distilled water failed because the particles adsorbed strongly to the glass plate. This adsorption was prevented by the addition of a detergent (experiment 1: 0.1 gm/liter Tide soap; experiments 2–4: 0.1 gm/liter linear alkyl benzenesulfonate, Soap and Detergent Association, N.Y., LAS lot no. 1-1). In examining the particles separated in experiment 1 we noted the presence of a large number of bacteria. Bacterial growth was prevented in the remaining experiments by the addition of 0.2 gm/liter merthiolate (Lilly). The solutions were filtered through a 0.45-μ Millipore filter. The fluid density was 0.998 gm/cm^3 (25°C).

Apparatus: The trough shown in Figure 1 was used on an ordinary table in a room reasonably free from vibration or large temperature gradients. The layer of fluid on the black plate was about 0.15 cm deep, 15.3 cm wide, and 50 cm long.

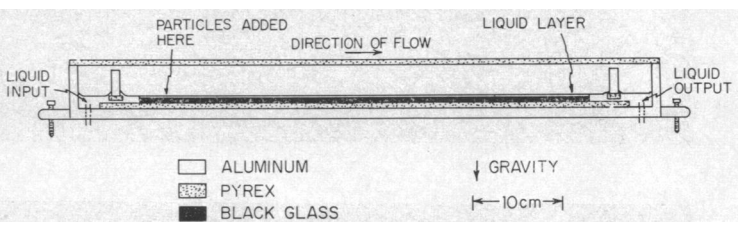

FIG. 1.—A schematic side view of the trough.

In experiments 1 and 2 this plate was Pyrex painted on the under side (Krylon flat black sealed with Dupont Vydax fluorocarbon); in experiments 3 and 4 we substituted a sheet of black float glass (Pilkington Brothers, Toronto). The black plate made it possible to photograph the particles by scattered light. The trough was sealed with a silicone rubber adhesive (G. E. RTV-102). Two aluminum and glass partitions, shown at either end of the trough in Figure 1, divided the apparatus into three chambers. Gaps under these partitions (0.015 cm thick) provided the high resistance necessary to ensure a flow uniform from one side of the trough to the other (except near the edges). Fluid was pumped out of the output chamber and back into the input chamber with a constant-rate roller pump (Holter Model RA034 with a 2 rpm synchronous motor and a PT025 pumping tube). Under these conditions the fluid depth was constant as long as the fluid volume remained constant. A glass cover was used to prevent evaporation, but it was removed when the spots were photographed, so the fluid level changed somewhat. In experiment 4 an additional sheet of Pyrex (not shown in Fig. 1) was mounted 0.159 cm above the black plate on spacers at the edges of the trough, and the fluid was made to flow between.

Procedure: Before running an experiment we thoroughly cleaned the black plate, using both fuming nitric acid and 10 M sodium hydroxide. If the surface was not clean, the spots tended to streak. The apparatus was leveled, the pump was allowed to transfer fluid into a tared vial for a timed interval, and drops containing a few hundredths of a milliliter of each particle preparation were added. The particles were allowed to sediment about eight hours. In experiments 3 and 4 a drop of a fluorescein solution (8 gm/liter) was then added. Its rate of travel was a measure of the average fluid velocity. The flow was turned on and the

spots were photographed about every 24 hours. We mounted a Polaroid-Tektronix oscilloscope camera on a stand which could be moved along a scale fixed to the apparatus. When the pictures were taken, the fluid was illuminated obliquely with two masked incandescent Lumiline bulbs positioned along the sides of the trough. The photographs were measured with the aid of a Polaroid transparency of a piece of graph paper. When the run was over, the fluid depth was measured again, so that some correction could be made for evaporation (experiments 2 and 3). Particle samples were collected with a dropper, centrifuged 15 minutes at 170 g, and resuspended for photomicrography in a dilute solution of polyethylene glycol (mol wt 20,000) sufficiently viscous to reduce Brownian motion.

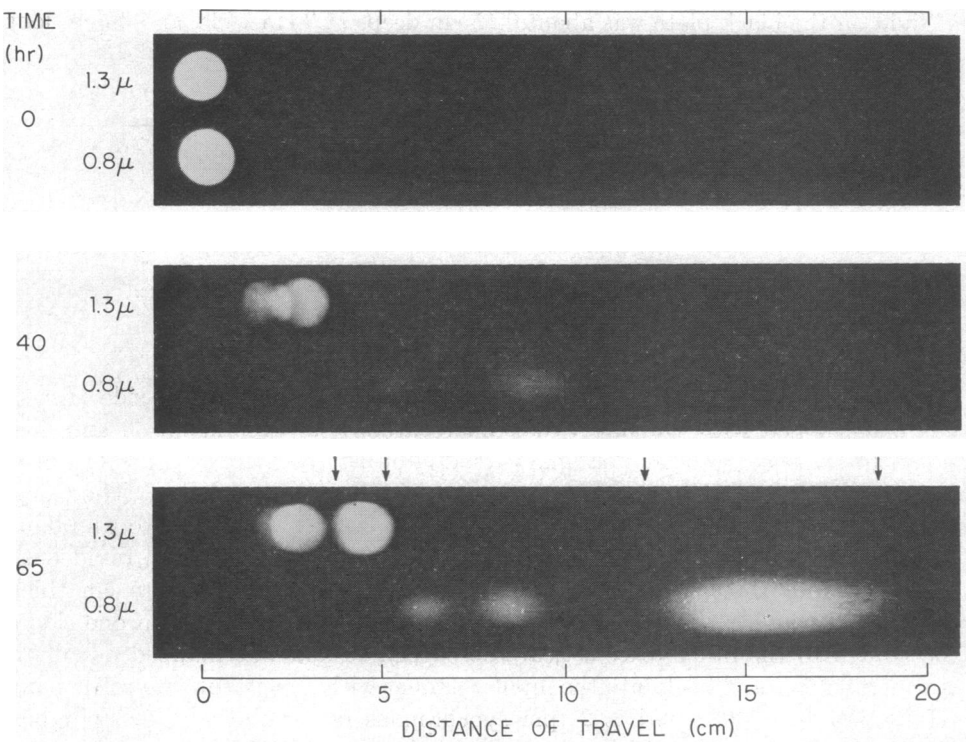

FIG. 2.—A montage of photographs taken during experiment 3 showing different stages in the separation of monomers, dimers, and trimers. The arrows indicate the spot widths predicted by the theory. The 40-hr photograph is underexposed.

Results and Conclusions.—The particles were not monodisperse: To our surprise, both the 0.8-μ and the 1.3-μ diameter particles separated into spots which traveled with velocities in the ratios of about $1 : {}^1/_2 : {}^1/_3$. The separation is evident in Figure 2, which shows photographs taken during experiment 3. This result implies that the original suspensions were not monodisperse but were in fact composed of single spheres (monomers), and of spheres stuck together in twos and threes (dimers and trimers). We confirmed this interpretation by examining the particles obtained from the different spots by phase contrast microscopy. Figure 3 shows micrographs of the 1.3-μ particles harvested from the final spots of experiment 3.

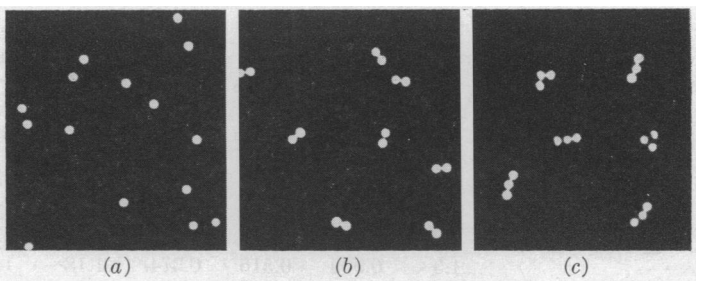

FIG. 3.—Photomicrographs of the 1.3-μ particles harvested in experiment 3 showing monomers (a), dimers (b), and trimers (c).

The monomer and dimer samples were essentially pure, but the trimer material contained a number of dimers and monomers. We consider these contaminants to be largely broken trimers, since their number depended critically on how gently the preparation was handled. Evidently the aggregate particles are stable during the flow experiment, since the spots remain discrete.

The upper surface of the fluid did not move: The velocity at which a spot travels depends on whether the upper surface of the fluid is free or fixed, that is, on whether the flow profile is "1-wall" or "2-wall."[4] For a given liquid depth and pumping rate the velocity near the bottom of the fluid is twice as great for the 2-wall profile as it is for the 1-wall profile, and particles near the bottom travel twice as fast. We had expected that the flow profile in experiments 1–3 would be 1-wall, but we found that the spots moved at the 2-wall rate. We also noticed that dust on the surface of the liquid remained nearly stationary. It thus appeared that the upper surface of the liquid was fixed. To find out whether this was true, we repeated the experiment under conditions in which the surface was known to be fixed: we covered the liquid with a second sheet of glass (experiment 4). Allowing for a slight difference in fluid depth, we found that the spots moved at the velocities observed before. We believe that the surface of the liquid in experiments 1–3 was held in place by a detergent monolayer. Monolayers of this kind are known to form at liquid-air interfaces. They are in fact strong enough to resist the viscous drag of the liquid. Langmuir's measurements,[5] for example, show that they can support surface pressures greater than 50 dynes/cm; the pressure on our monolayer was only 10^{-2} dynes/cm.

The spot velocities and widths were in reasonable agreement with theory: For a 2-wall profile, the theory[6] predicts that a particle will travel with the average velocity

$$\bar{v}_{\text{predicted}} = (6v_a h_s/h_0)(1 - h_s/h_0), \tag{1}$$

where v_a is the average velocity of the fluid, h_0 is the depth of the fluid, and $h_s \ll h_0$ is the scale height of the particle. For a sphere of diameter d and density ρ in a fluid of density ρ_0,

$$h_s = 6kT/\pi d^3(\rho - \rho_0)g, \tag{2}$$

where k is Boltzmann's constant, T is the absolute temperature, and g is the acceleration of gravity. For the 0.8-μ monomer, $h_s = 29.8\ \mu$; for the 1.3-μ monomer,

TABLE 1

VALUES OF THE EXPERIMENTAL PARAMETERS AND A COMPARISON WITH THEORY

Expt.	Length of expt. (hr)	Fluid depth h_0 (mm)*	Av. fluid velocity v_a (mm/hr)†	Particle type (μ)	Average Particle Velocity $\bar{v}_{obs.}$(mm/hr)‡ Mono-mers	Dimers	Trimers	$\bar{v}_{obs.}/\bar{v}_{pred.}$ Mono-mers	Dimers	Trimers
1	86	1.40 ± 0.10	23.2 ± 2.0	0.8	2.66	1.48	0.969	0.93	1.02	0.99
				1.3	0.738	3.94		1.11	1.18	
2	76	1.35 ± 0.04	25.8 ± 0.7	0.8	3.18	1.81	0.846	0.97	1.08	1.17
				1.3	0.909	0.515	0.374	1.18	1.34	1.46
3	125	1.47 ± 0.04	20.7 ± 0.5	0.8	2.46	1.29	0.738	1.02	1.05	1.11
				1.3	0.656	0.383	0.292	1.16	1.35	1.92
4	95	1.59 ± 0.02	19.8 ± 0.3	0.8	2.19	1.16		1.02	1.06	
				1.3	0.586	0.322		1.17	1.28	

* Expts. 1–3: the mean of the initial and final fluid depths with an uncertainty of 15% of the difference. Expt. 4: the uncertainty is the estimated error in the spacing of the plates.

† Expts. 1–2: computed from the pumping rate and the width and depth of the fluid. Expts. 3–4: the measured fluorescein velocity.

‡ We estimate the uncertainty in the velocity measurements to be about 3% for the monomers and dimers, about 5% for the trimers.

$h_s = 6.76\ \mu$. The scale heights of the dimers and trimers are $^1/_2$ and $^1/_3$ as large, respectively.

Table 1 gives the measured average particle velocities, $\bar{v}_{\text{observed}}$, for experiments 1–4. The measured values of h_a and v_a are also given; these were used to compute $\bar{v}_{\text{predicted}}$ from equation (1). The ratio $\bar{v}_{\text{observed}}/\bar{v}_{\text{predicted}}$ is a measure of the agreement between the experiment and the theory; it is tabulated in the last three columns. The absolute values of these ratios are subject to the uncertainties in h_0 and v_a, but the relative values of the ratios in a given experiment are not. The agreement between experiment and theory is quite good for the lightest particles; however, the heavier particles traveled more rapidly than predicted. A number of effects not included in the theory may be responsible for this; we suspect that the most important of these involves the failure of the assumption that particle size is small compared to scale height.

The monomer diffusion constants can be computed from the Einstein-Smoluchowski relation and Stokes' law; for the 0.8-μ monomers, $D = 5.4 \times 10^{-9}$ cm^2/sec, and for the 1.3-μ monomers, $D = 3.3 \times 10^{-9}$ cm^2/sec. These values can be used with the scale heights to estimate the theoretical half-intensity points of the monomer bands.[7] These points are indicated by arrows in the 65-hour photographs of experiment 3, Figure 2. The heavier particles spread less, even though their diffusion constants are smaller, because their Boltzmann distributions are not as high. Spreading due to diffusion in a direction parallel to the length of the trough was relatively small, as expected; this is evident in Figure 2, since the spots did not spread much sideways.

Summary.—We have described a series of experiments which utilize the earth's gravitational field to test a new method for separating particles according to mass. Polystyrene latex particles were separated into discrete bands composed of monomers, dimers, and trimers. The band velocities and widths were in reasonable agreement with theory. The gravitational method should be generally useful in the effective mass range spanned by the latex particles, 10^{-14} to 10^{-13} gm. Particles of widely different absolute masses may be brought within this range by varying

the density of the fluid. If surfaces can be found to which particles do not adsorb in the absence of detergents, the method might be of use to biologists, for example, in separating chromosomes or synchronizing bacterial populations.

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[2] *Ibid.*, in press.
[3] Bradford, E. B., and Vanderhoff, J. W., *J. Appl. Phys.*, **26**, 864 (1955).
[4] Ref. 1, Fig. 1.
[5] Langmuir, I., *J. Am. Chem. Soc.*, **39**, 1848 (1917).
[6] Ref. 1, equations (1), (3), and (4).
[7] Ref. 1, equation (27), with an approximate allowance for the initial widths of the spots.