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J. C. Giddings^a; G. Karaiskakis^{ab}; K. D. Caldwell^a

^a DEPARTMENT OF CHEMISTRY, UNIVERSITY OF UTAH SALT LAKE CITY, UTAH ^b Department of Chemistry, University of Patras, Patras, Greece

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Density and Particle Size of Colloidal Materials Measured by Carrier Density Variations in Sedimentation of Field-Flow Fractionation

J. C. GIDDINGS, G. KARAIKAKIS*, and K. D. CALDWELL

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF UTAH
SALT LAKE CITY, UTAH 84112

ABSTRACT

A new approach has been developed to yield both the particle density and particle mass (or size) of colloidal materials using a sedimentation field-flow fractionation system. The method involves varying the density of the carrier fluid, in the present case using aqueous sucrose solutions. Five polystyrene latex bead samples with different diameters were subjected to the procedure. These provided experimental density values all within 0.4% of the manufacturer's value. Particle sizes determined experimentally agreed quite well both with manufacturer's values and with diameters obtained from previous FFF experiments using a carrier of constant density. The method has considerable promise for characterizing particle mass (size) and density parameters in complex colloidal materials.

INTRODUCTION

Retention in sedimentation field-flow fractionation (sedimentation FFF) can be rigorously related to particle mass, particle density, carrier density, field strength, channel width, and temperature. All of these parameters can be controlled or directly determined as an essential part of the FFF system except the particle parameters of mass and density. A single retention measurement will then provide one relationship whose only unknowns

are particle mass and density. If density is known independently, the single measurement will serve to determine particle mass and thus particle volume and size. However, if density is not known, additional information is needed for particle characterization. A second measurement of retention, this time at a different carrier density, will provide another relationship with the same unknowns so that we now have two equations to fix the value of the two unknowns. In practice, of course, it is best to determine retention at a number of carrier densities to obtain the most accurate possible values of both particle mass and density. In this paper we develop a graphical procedure exploiting this concept. The method is tested using several well characterized samples of polystyrene latex beads.

The theoretical basis for the influence of particle mass and field strength on retention in sedimentation FFF has been discussed in earlier papers (1,2). Many subsequent experiments have been carried out, all at constant carrier density, for particles of a known or assumed density. The measurements have verified theoretical predictions (1,3), and the equations have been used to conveniently determine the molecular weights of the T2 virus (3) and the gypsy moth NPV (nuclear polyhedrosis) virus (4). We now seek to develop the means to characterize particle density as well as mass or molecular weight.

THEORY

Retention Equations

Retention in sedimentation FFF is a function of a dimensionless parameter λ which approximately represents the ratio of the mean thickness of the solute layer to the channel width w (5). The fundamental expression for λ is

$$\lambda = \frac{D}{w|U|} \quad (1)$$

where D is the diffusion coefficient of the solute and $|U|$ is the absolute velocity of the solute induced by the centrifugal field. The level of retention can be obtained from the basic retention equation (5)

$$R = 6\lambda[\coth(1/2\lambda) - 2\lambda] \quad (2)$$

where retention ratio R is

$$R = V^0/V_r \quad (3)$$

in which V^0 is the column void volume (or elution volume of a non-retained solute peak), and V_r is the retention volume of the particle or solute fraction. Under conditions of high retention Eq. (2) assumes the simple form (5)

$$R = 6\lambda \quad (4)$$

The substitution of $|U| = |F|/f$ and $D = R/T/f$ into Eq. (1) yields (5)

$$\lambda = \frac{R/T}{|F|w} \quad (5)$$

which is the ratio of thermal energy R/T , to the energy change experienced by the solute traveling across the full width of the channel. Here F is the effective force induced by the field on a mole of solute, f is the molar friction coefficient, R is the gas constant, and T is the absolute temperature. The absolute value of the force F acting on Avogadro's number of particles is

$$|F| = MG|\Delta\rho|/\rho_s$$

where M is particle molecular weight, G is the field strength in units of acceleration, ρ_s is the solute (particle) density, and $|\Delta\rho|$ is the absolute value of the difference between ρ_s and carrier density ρ . Substitution of this relationship into Eq. (5) gives

$$\lambda = \frac{R/T}{MGw(|\Delta\rho|/\rho_s)} \quad (6)$$

Equation (6) demonstrates the relationship between retention ($\lambda \approx R/6$) and the difference $\Delta\rho = \rho_s - \rho$ between solute and carrier density. By changing the density of the carrier this equation can

provide both the solute density ρ_s and the particle mass or size. The procedure is as follows.

Density and Particle Size Determination

The difference between solute (ρ_s) and carrier (ρ) density can be positive or negative; we will distinguish these two cases in our discussion.

In the first case, when $\rho_s < \rho$, we have from Eq. (6)

$$\lambda = \frac{R/T}{MGw \frac{\rho - \rho_s}{\rho_s}} = \frac{R/T}{MGw \frac{\rho}{\rho_s} - MGw} \quad (7)$$

Taking the inverse form of Eq. (7), we obtain

$$\frac{1}{\lambda} = \frac{MGw}{R/T} \cdot \frac{\rho}{\rho_s} - \frac{MGw}{R/T} \quad (8)$$

or

$$\frac{R/T}{\lambda Gw} = M \frac{\rho}{\rho_s} - M \quad (9)$$

Substituting the symbol M' for the left hand term of Eq. (9) we get

$$M' = M \frac{\rho}{\rho_s} - M \quad (10)$$

or

$$\rho = \rho_s + \frac{\rho_s}{M} M' \quad (11)$$

in which M' is a measurable quantity (through retention parameter λ) equal to the "effective molecular weight" of the solute or particulate material

$$M' = \frac{R/T}{\lambda Gw} = \frac{|F|}{G} = \frac{M|\Delta\rho|}{\rho_s} \quad (12)$$

For the case in which the "solute" is a colloidal particle, emulsion droplet, etc., molecular weight M can be replaced by the particle volume V_s by using $M = V_s \rho_s N$, where N is Avogadro's

number. In the special case of spherical beads (as used here), the equivalent molecular weight M can be related to bead diameter d by (6)

$$M = (1/6) N \pi \rho_s d^3 \rightarrow 0.31534 \rho_s d^3 \quad (13)$$

where in the latter expression, ρ_s is the solute density in g/ml and d is the particle diameter in Angstroms. Equation (11) can be converted to

$$\rho = \rho_s + \frac{1}{0.31534 d^3} M' \quad (14)$$

From this equation we deduce that a plot of carrier density ρ versus M' is expected to give a straight line with a positive slope equal to $1/0.31534 d^3$, from which we can determine particle diameter, and with an intercept equal to solute density, ρ_s

$$\text{Slope} = \frac{1}{0.31534 d^3} \quad (15)$$

and

$$\text{Intercept} = \rho_s \quad (16)$$

In the second case, when $\rho_s > \rho$, following the same procedure, Eq. (11) gives

$$\rho = \rho_s - \frac{1}{0.31534 d^3} M' \quad (17)$$

Equation (17) shows that a plot of ρ versus M' should give a straight line with a negative slope. As in the previous case, the slope gives the diameter of the solute particle and the intercept gives the solute density.

EXPERIMENTAL

A description of the sedimentation FFF column and the method of operation have been presented (7). The channel dimensions in this study were $47.5 \times 0.0254 \times 1.0$ cm. Channel void volume as measured by the elution of a void peak was 0.90 ml.

Carrier flow was provided by a Minipuls 2 peristaltic pump from Gilson Co. A UV detector (Laboratory Data Control) immediately after the channel exit and an Omniscribe recorder (Houston Instruments) provided fractograms of the eluted colloids.

The nearly monodisperse polystyrene beads (Dow Diagnostics Co.) had reported diameters in Angstrom units of 2200 ± 65 , 3570 ± 56 , 4810 ± 18 , 6200 ± 76 and 7600 ± 46 . The corresponding particle diameters obtained in an unpublished work by experimental sedimentation FFF elution volumes were 2270, 3570, 5240, 6200, and 7500, respectively.

Carrier liquids used were several sucrose (from Amachem Co.) solutions in 0.1% FL-70 detergent (from Fisher Scientific Co.), with different concentrations providing different densities (Table 1). The densities were determined by weighing known volumes of

TABLE 1

Concentrations and Corresponding Densities of Aqueous Sucrose Solution

%* by weight	density (g/cm ³)
0.0	1.000
3.0	1.009
4.5	1.015
6.0	1.021
27.0	1.110
29.0	1.121
31.0	1.130
35.0	1.149

*The percentage by weight entries are approximate values.

the liquid. For this purpose, a 50 ml volumetric flask was calibrated by filling with doubly distilled water and weighing. (The density of distilled water is accurately known at the given temperature.) A Mettler Model P 162 balance with an accuracy of 0.001 g was used to weight the flask.

RESULTS AND DISCUSSION

Densities and particle diameters for the five different polystyrene latex bead samples have been determined by the above procedure using data from nine to fourteen experimental runs at different carrier densities for each sample. Experimental values for retention ratio R and values of M' calculated from R using Eq. (4) and Eq. (12) are reported as functions of solvent density in Table 2. The results in the table are divided into the two categories discussed earlier, the first in which particle density is less than that of the carrier ($\rho_s < \rho$), and the second in which the densities are reversed ($\rho_s > \rho$).

In Figures 1 and 2 we present plots of the ρ versus M' values in Table 2 for two of the polystyrene latex bead samples, those with nominal particle diameters of 3570 and 4810 Angstroms, respectively. For both samples, the points for both the upper ($\rho_s < \rho$) and lower ($\rho_s > \rho$) branches fall on straight lines, verifying our theoretical conclusions. From these plots (and the plots, not shown here, for the other latices), we can acquire particle

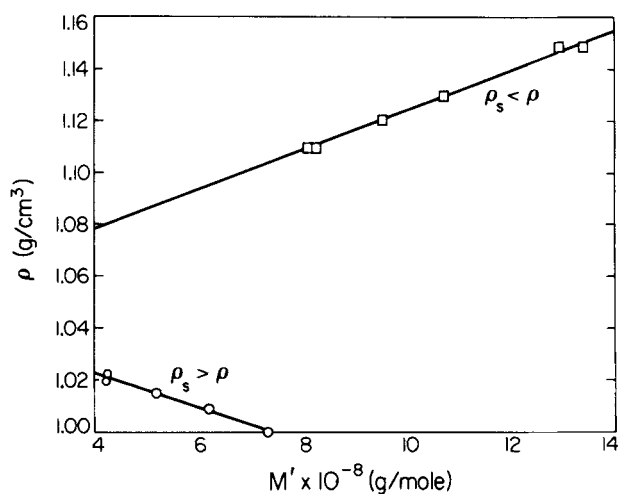


FIGURE 1. Plot of ρ versus M' for polystyrene latex beads of nominal diameter 3570A.

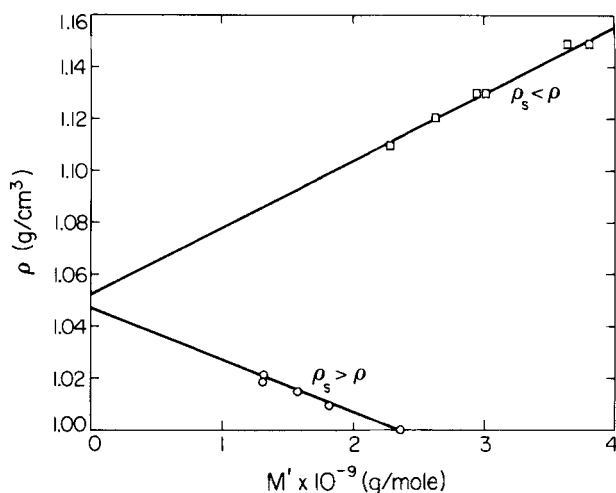


FIGURE 2. Plot of ρ versus M' for polystyrene latex beads of nominal diameter 4810A.

size and density parameters using Eqns. (15) and (16).

The density of the polystyrene bead samples, obtained from the intercept of the ρ versus M' plots (Eq. (16)), are shown in Table 3. The results show that the low and high density branches converge rather closely, as expected. There is no systematic difference between them, as indicated by the closeness of the average densities obtained from the two branches, 1.051 and 1.050, respectively.

Table 3 also shows the apparent error in the mean density values, taking as the "theoretical" value the density given by the manufacturer, 1.05 g/ml. The mean density $\bar{\rho}_s$ signifies the average taken from the intersection of the two straight lines for any given sample in the plots of ρ versus M' (Figures 1 and 2). Percentage errors of between 0.00% and 0.038% show that the FFF method gives excellent agreement with independently acquired results for the densities of these spherical colloidal materials.

The calculated particle diameters for both density branches are shown in Table 4. Table 4 also gives the mean values \bar{d} for particle diameters obtained from averaging the two branches,

TABLE 2

Experimental Retention Ratio, R, and the Derivative Parameter, M' , for Polystyrene Latex Beads at Different Carrier Densities. Bead Diameter d is Value Provided by Manufacturer

d (nominal) (A)	$\rho_s < \rho$			$\rho_s > \rho$		
	R	$10^{-9} \times M'$ (g/mole)	ρ (g/ml)	R	$10^{-9} \times M'$ (g/mole)	ρ (g/ml)
2200	0.111	0.245	1.110	0.120	0.226	1.000
	0.103	0.263	1.121	0.120	0.226	1.000
	0.096	0.283	1.130	0.145	0.198	1.009
	0.073	0.377	1.149	0.164	0.168	1.015
	0.074	0.377	1.149			
3570	0.059	0.821	1.110	0.066	0.731	1.000
	0.060	0.804	1.110	0.079	0.619	1.009
	0.051	0.954	1.121	0.093	0.519	1.015
	0.045	1.072	1.130	0.111	0.423	1.021
	0.036	1.340	1.149	0.111	0.423	1.021
	0.037	1.297	1.149			
	0.063	2.287	1.110	0.062	2.363	1.000
4810	0.054	2.626	1.121	0.077	1.818	1.009
	0.048	2.954	1.130	0.091	1.576	1.015
	0.047	3.030	1.130	0.106	1.313	1.021
	0.039	3.636	1.149	0.105	1.313	1.021
	0.037	3.833	1.149			
	0.083	3.309	1.110	0.076	3.649	1.000
6200	0.083	3.309	1.110	0.098	2.895	1.009
	0.073	3.797	1.121	0.113	2.438	1.015
	0.064	4.211	1.130	0.129	2.106	1.021
	0.064	4.211	1.130	0.129	2.106	1.021
	0.050	5.581	1.149			
	0.050	5.581	1.149			
	0.080	5.402	1.110	0.069	6.580	1.000
7600	0.080	5.402	1.110	0.069	6.580	1.000
	0.068	6.294	1.121	0.087	5.170	1.009
	0.061	7.190	1.130	0.087	5.170	1.009
	0.061	7.190	1.130	0.101	4.258	1.015
	0.049	8.831	1.149	0.118	3.619	1.021
	0.049	8.831	1.149	0.118	3.619	1.021

together with two measures of percentage deviation in d , one with reference to the "nominal" d given by the manufacturer, and the other with reference to d values from previous but yet unpublished sedimentation FFF experiments at constant carrier density using an assumed particle density of 1.05 g/cm. We note that the deviations from the manufacturer's diameters are generally larger

TABLE 3

Densities of Polystyrene Beads Determined from the Intercepts of Plots of ρ vs M' . The Last Column Tabulates the Deviation of the Mean Measured Density, $\bar{\rho}_s$, from the Manufacturer's Value, 1.05 g/cm³.

d (nominal) (A)	Densities of Polystyrene Beads (g/cm ³)			$\frac{\bar{\rho}_s - \rho_{\text{theor}}}{\rho_{\text{theor}}} \times 100$
	ρ_s (for $\rho_s < \rho$)	ρ_s (for $\rho_s > \rho$)	$\bar{\rho}_s$	
2200	1.050	1.057	1.054	+0.38
3570	1.048	1.050	1.049	-0.09
4810	1.052	1.047	1.050	0.00
6200	1.056	1.049	1.053	+0.29
7600	1.049	1.046	1.048	-0.19
Average	1.051	1.050	1.051	

TABLE 4

Diameters of Polystyrene Beads Determined from Retention Experiments in Sedimentation FFF by Varying Solvent Density. Values in the Column Designated "FFF" were Obtained from Unpublished FFF Retention Experiments in which the Density was Assumed to be the Manufacturer's Value, 1.05 g/cm³.

d = Diameter of Polystyrene Beads (A)					% Deviation in \bar{d} from:	
Nominal	FFF	$\rho_s < \rho$	$\rho_s > \rho$	\bar{d}	nominal	FFF
2200	2270	2290	2330	2310	+5.00	+1.76
3570	3570	3460	3620	3540	-0.84	-0.84
4810	5240	4960	5380	5170	+7.84	-1.34
6200	6200	5740	6150	5950	-4.03	-4.03
7600	7500	6550	7670	7110	-6.45	-5.20

than those from the previous FFF measurements. We think it likely that our FFF-derived values are more reliable than the manufacturer's values. This matter will be discussed in another publication.

We note also that the lower branch ($\rho_s > \rho$) yields d values that are systematically higher than the upper branch ($\rho_s < \rho$). This may be due to some slight differences in the upper (inner) and lower (outer) surfaces (perhaps in surface roughness) of the channel. However, the difference might also be due to the larger average $|\Delta\rho|$ values used with the upper branch. The larger particles, pushed closer to the wall because of larger $|\Delta\rho|$'s, would migrate slightly more rapidly than they should due to a small steric FFF contribution (8.9). This would reduce the measured M' value at high $|\Delta\rho|$'s, increase the slope of the ρ versus M' plots, and yield apparent d values of slightly smaller magnitude than the actual values. One problem with this explanation is that the retraction of the extreme points on the M' scale (more so on the upper than the lower branch) should tilt the lines to yield a smaller intercept for the upper than the lower branch. The intercepts (densities) of Table 3 clearly do not follow this trend for the larger particles. Obviously, more work is needed to find the source of this discrepancy.

Despite small, systematic differences in d values, the present FFF method appears to yield quite accurate values for both the density and size of colloidal particles. The method should work as well for nonspherical particles as for spheres since retention hinges directly on particle mass (and density), and only on particle dimensions as a secondary matter. Consequently, we feel that this approach has considerable promise in characterizing both the particle mass (or some dimension) and density of a large number of complex biological, environmental, and industrial colloidal materials.

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