DENSITY GRADIENT SELF-GENERATION BY OSMOCENTRIFUGATION APPLICATION TO THE STUDY OF LATEX PARTICLES

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ABSTRACT

Density gradients obtained by centrifuging Ficoll solution in dialysis cells are used in fractionation and latex particle size determination (by sedimentation coefficient calculations). Mixed polymer latexes are layered over these gradients and centrifuged again, leading to separate latex bands, from which polymer may be collected and further analysed by standard (IR, NMR, etc.) procedures. It is demonstrated that the supernatant of a coagulated polystyrene latex contains all the possible oligoaggregates: doublets, triplets, etc. up to hexuplets. Zone migration rates in the osmocentrifugation experiment are faster than in standard centrifugation tubes.

INTRODUCION

Placing a solution of a colloidal sol within one compartment of a dialysis cell, fitted with a vertical membrane (the other side of which contains solvent), leads to a liquid circulation pattern, the net result of which is faster approach to sedimentation equilibrium. This phenomenon has been described in the literature[1] and it was previously used to do various kinds of sedimentation experiments concentration of proteins and polysaccharides[2], under low centrifugation speeds, macromolecular solute fractionation[3]; polymer MW determination[4]; colloidal particle size and repulsion potential determination[5].

A more recent application of osmocentrifugation is the generation of density gradients, suitable for cell fractionation[6] and particle characterization[7], much more readily than the usual centrifugation experiments.

Polymer latexes are importants components of end-use products, as well as useful intermediates in the plastics and rubber industry[8]. Their characterization requires the evaluation of many properties, among these, particle size distribution and / or particle density can be determined by electron microscopy[9], light scattering[10], flow ultramicroscopy[11], ultracentrifugation[12] and in Couter counters[13]. Each of these methods has advantages and disadvantages. On the other side, latex fractionation is a more difficult matter, particularly at the preparative scale.

Sedimentation in density gradients has been used for density determination in isopycnic experiments, in the density gradient columns, suitable for large ($\phi > 1$mm) polymer bodies. Both isopycnic and sedimentation velocity determinations, in the ultracentrifuge, are widely used in biological particle determination[14].

H. Lange worked out a fast density gradient centrifugation technique and used it with polymer latexes: a low-density liquid is layered over a high-density liquid (which contains the sample under study) in a synthetic-boundary cell, in the analytical ultracentrifuge; the gradient is formed by diffusion and isopycnic equilibrium is reached in short times. This method allows separation of mixed latexes and their chemical characterization[15,16].

W. Maechtle[17] did also use dynamic gradients, formed by osmocentrifugation of Percoll in H_2O, D_2O or MeOH. This technique was succesfully used in the study of fine particles (polymer latexes, microcrystals and bacteria cells) down to 300 nm diameter.

In this work we describe the generation of density gradients suitable for polymer latex fractionation, by size and chemical composition (density).

EXPERIMENTAL

Ficoll 400, Percoll and Density Marker Beads were obtained from Pharmacia (Sweden). Polystyrene latex beads were from SIGMA; three different sizes were used: 0.109, 0.305 and 0.460 μm diameter. Other reagents were analytical grade.

Osmocentrifugation cells[2,6] were made out of acrylic sheets or glass fiber-reinforced polyester. Typical dimensions were (7.5x3.5x0.7 cm) (Figure 1). Cellulose acetate membranes were cast following the procedure described by Nunes et al.[18], from a solution containing 10 g of cellulose acetate, 38 ml acetone, 35 ml acetic acid and 21 ml water.

Centrifugation runs were performed in a RC-3B Sorvall Refrigerated Centrifuge, with a swinging-bucket rotor. Density measurements were made in a PAAR DMA 600/602 instrument and (indirectly) with a Bausch & Lomb Abbe Refractometer.

The osmocentrifugation cells were assembled and the compartment on one side of the membrane was filled with gradient-former solution, the other with solvent. The cells were placed in the centrifuge swinging bucket and spun for the desired time, when the density gradient is obtained. The sample to be fractionated can then be layered over the solution and the cell is spun again. Latex band movement can be monitored visually; cell contents can be assayed by drawing solution aliquots, from given heigths. This is conveniently done with a long-needle attached to a syringe or to tubing mounted on a peristaltic pump.

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RESULTS

Density gradient self-generation, by osmocentrifugation. Density gradients are generated by centrifugation of Percoll, Ficoll and Ficoll-Sucrose aqueous solutions in one of the compartments of a dialysis cell, while the other compartment contained the solvent, either pure water or aqueous sucrose solution. After the given centrifugation times cell contents are withdrawn and the density of aliquots are determined. Representative data are in Figure 2 and Table 1.

Polystyrene latex fractionation in Ficoll density gradients. Ficoll density gradients were self-generated by osmocentrifugation of an aqueous solution (starting density 1.024 g/ml) at 3000 rpm, 20°C, for 2 hours. A layer of PS latex (0.2 ml, 2% w/w) was placed over the density gradient column and the cell was spun for another 4 hours. Cell content fractions were taken at various cell heights, the refractive indexes and turbidities were measured. The results are given in Figure 3. Well-resolved latex band formation is observed both in runs performed with monodisperse latex samples and in runs performed with latex admixture.

Density gradient stability in the osmocentrifugation cell. To determine density changes in the osmocentriugation cell, runs were performed, in Ficoll gradients obtained by centrifuging a solution for 2 hours, in the osmocentrifugation cell (3000 rpm, 20°C). A mixture of PS latexes of three different sizes was layered at the performed gradient top, together with density marker beads. The cells were then centrifuged (3000 rpm, 20°C); both latex band and marker bead displacement were followed. Figure 4 shows a plot of latex band/bead position, as a function of time. Some conclusions can be drawn: i) bead position changes are noticeable, up to 6 hours; minor changes are detected after this time; ii) latex bands displace independently, but the larger particles bands merge after 52 hours, as expected considering that isopycnic equilibrium is approached; the lighter latex band smeared out and could not be followed beyond ca. 28 h. These results show that latex band migration occurs in a downwards moving, changing-density medium for the first hours and in an unchanging inmobile medium after this initial period.

Latex band migration rates in osmocentrifugation and in normal centrifugation. A density gradient was performed by centrifugation of a Ficoll 8% (w/w) and Sucrose 4% (w/w) solution (6.5 cm tall) within a dialysis cell. The gradient was then transferred to a glass tube (giving a 6.1 cm tall liquid column) and overlayed with 0.2 ml of 0.2% latex (mixture of 0.305 and 0.460 μm diameter

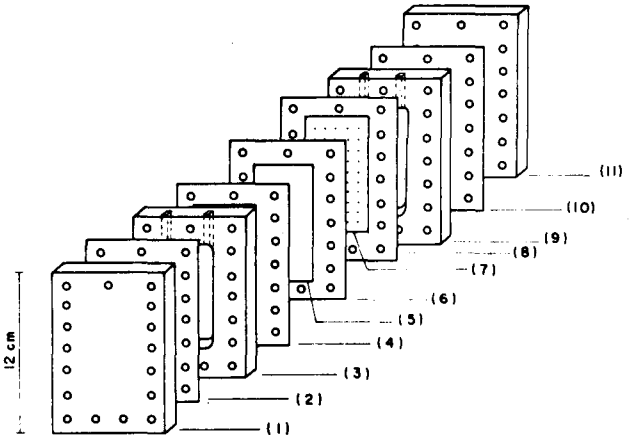

Fig. 1 – Osmocentrifugation cell (1, 3, 9, 11) acrylic sheet; (2, 4, 6, 8, 10) polyethylene sheet; (5) membrane; (7) Ni porous sheet.

TABLE 1

Characteristics of density gradients obtained by osmocentrifugation.

Solution – initial density	Running Conditions t (min)/ w (rpm)	Density range (g/ml)	$\Delta\rho/\Delta r \cdot 10^5$ (g/cm^4)
Percoll – 1.12 g/ml	15 / 1000	1.07 – 1.17	8.0
	15 / 1500	1.06 – 1.16	15.0
	20 / 2500	1.02 – 1.19	30.0
	120 / 2500	1.00 – 1.25	70.0
Ficoll – 1.03 g/ml	60 / 1000	1.02 – 1.03	2.0
	120 / 1000	1.02 – 1.03	1.5
	60 / 2000	1.02 – 1.04	3.0
	120 / 2000	1.02 – 1.04	3.5
Ficoll / Sucrose – 1.13 g/ml	70 / 2000	1.10 – 1.12	3.3
	140 / 2000	1.11 – 1.13	2.0

$\Delta\rho/\Delta r$: density gradient at half-heigth.

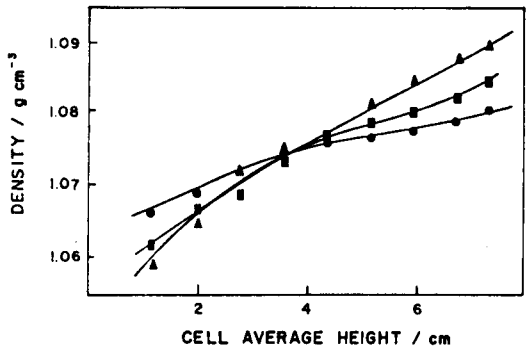

Fig. 2 – Ficoll-sucrose density gradients obtained by osmocen trifugation of an 8% Ficoll, 14% sucrose solution starting density 1.08 g/ml. Running conditions (●) 20°C, 1h, 1000 rpm; (■) 20°C, 2 h, 2000 rpm; (▲) 20°C, 1h, 3000 rpm (each curve is the average of three experiment).

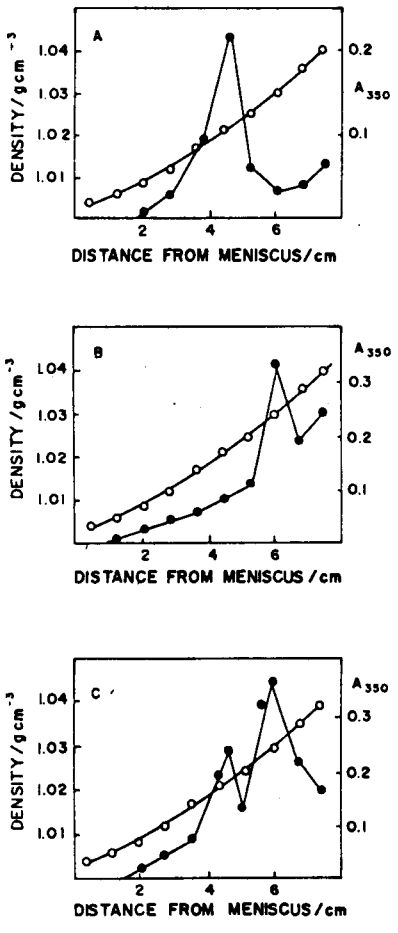

Fig. 3 – PS latex band formation in the osmocentrifugation cell. Latex particle diameter (A) 0.305 μm; (B) 0.460 μm; (C) 0.305 μm plus 0.460 μm. Runs at 3000 rpm, 20°C. Ficoll gradients were preformed, during 2 hours; sample zone were added and spun for another 4 hours.

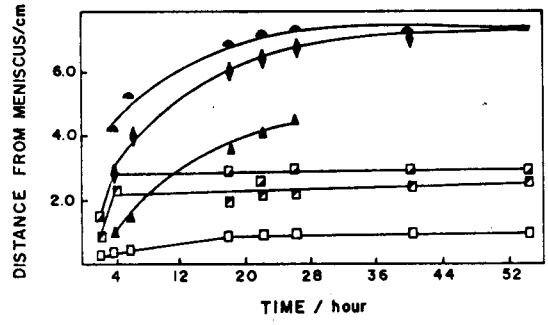

Fig. 4 – PS zone and density marker displacement in the osmocen trifugation cell, aas a function of time. 3000 rpm, 20°C. PS particle diameter (◓) 0.460 μm, (◆) 0.305 μm, (▲) 0.109 μm. Marker density: (□) 1.002); (◩) 1.008; (◪) 1.010 g/ml.

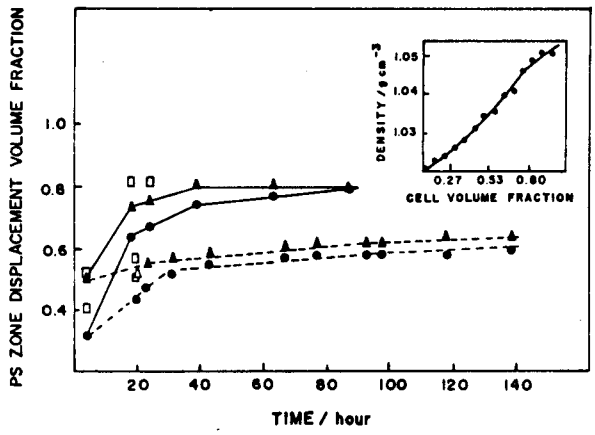

Fig. 5 – PS zone displicement in the osmocentrifugation cell (–) and glass tube (– – –), as a function of time. Runs at 3000 rpm, 20°C. PS particle diameter (▲) 0.460 μm; (●) 0.305 μm; (□) faint zones. The insert gives density gradient of Ficoll 8% (w/w) solution. Runns at 3000 rpm, 4 h, 20°C.

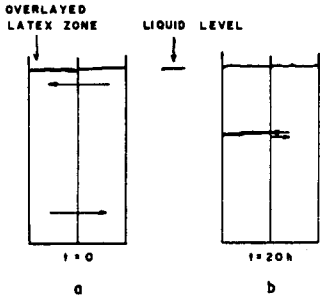

Fig. 6 – Proposed mecanism for faster zone migration in osmocen trifugation. There is an osmotic current at the zone bottom and a reverse osmotic at its top. The result is bulk Zone (latex + solute) movement, which does not exist in normal centrifugation.

particles). The glass tube was then centrifuged at 3000 rpm 20°C and the position of the bands was measured as a function of time. Another parallel experiment was performed in the same way but with a difference the PS latex was layered over the gradient within the same dialysis cell

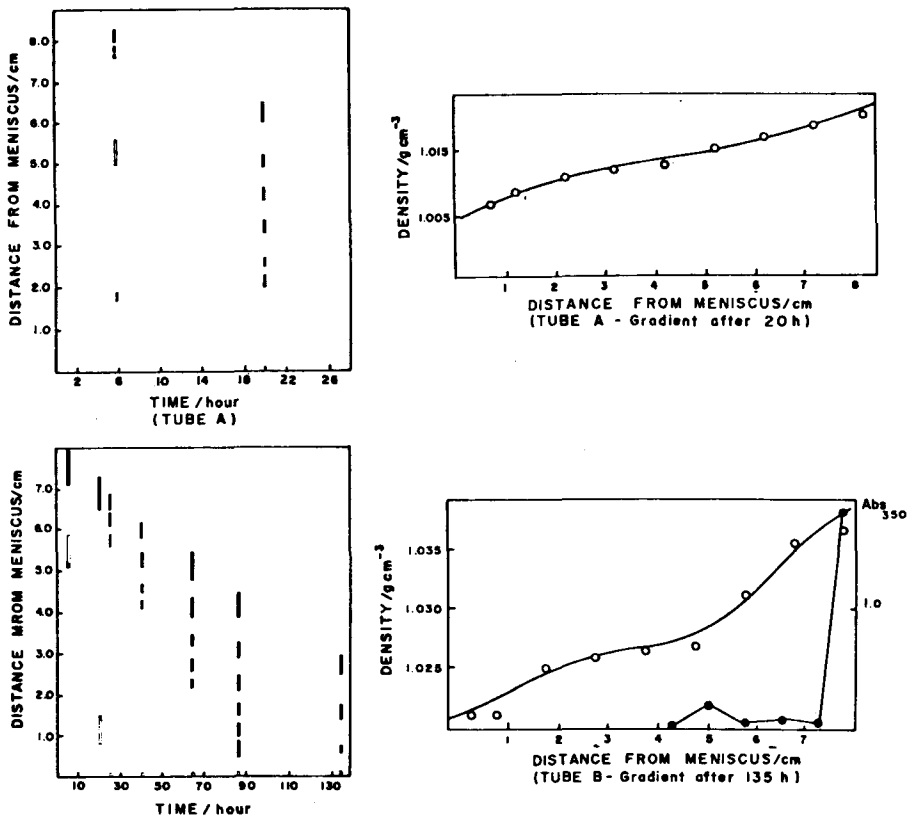

Fig. 7 – PS zone displacement in the centrifugation tube as a function of time. Running conditions: 3000 rpm, 20°C. Tube A: PS particle diameter (□) 0.305 μm and (■) 0.109 μm (supernatant of a coagulated sample). Tube B: PS particle diameter (□) 0.460 μm and (■) 0.109 μm (supernatant of a coagulated sample).

in which this was obtained, and spun. The results given in Figure 5 show that isopycnic equilibrium is reached at ca. 40 h, in the osmocentrifugation run but the latex zones are still well away from equilibrium in the glass tube, after 140 h. PS latex buoyant density was determined, 1.048 g/ml, in agreement with literature data. This proves that zone motion is faster in osmocentrifugation than in normal sedimentation.

We should recall that a steady density gradient is reached after a few hours osmocentrifugation. Consequently, faster zone displacement in the osmocentrifugation cell cannot be assigned to bulk, downwards liquid movement. We postulate that local osmotic and reverse-osmotic liquid currents are established above and bellow the zone, pushing it downwards and faster than by independent particle movement. See Figure 6.

High resolution latex fractionation. Ficoll gradients were generated by osmocentrifugation (2 h, 3000 rpm, 20°C) and transfered to centrifuge glass tubes (A and B) with a peristaltic pump. Tube A contained the lower density gradient (1.005–1.020 g/ml) and the tube B the denser one (1.020–1.038 g/ml). Density measurements taken before and after liquid transfer showed that the gradient is unaltered, when transfered. In tube A and B the supernatant of a coagulated sample was mixed with a noncoagulated sample and laid over the column. Centrifugation experiments presented in Figure 7 show that a further fine fractionation was obtained.

CALCULATION OF SEDIMENTATION COEFFICIENTS IN FICOLL GRADIENTS: THEORETICAL FOUNDATIONS.

The sedimentation rate of a particle in a liquid density gradient is a function of the amount and duration of the applied acceleration, the size and density of the particle, and the density and viscosity of the medium. In a homogeneous medium m, at temperature T, the sedimentation coefficient $s_{T,m}$ is given by the equation[19,20]

$$s_{T,m} = \frac{(dR/dt)}{w^2 R} \qquad (1)$$

where w is the angular velocity in radians by second, R is the distance from the rotor center to the particle, and dR/dt is the velocity of the particle.

The experimentally determined sedimentation coefficient, s is usually converted to a standard state defined as a coefficient in water at 20°C using the equation[19,20]

$$s_{20,w} = s_{T,m} \frac{\eta_{T,m}(\rho_p - \rho_{20,w})}{\eta_{20,w}(\rho_p - \rho_{T,m})} \quad (2)$$

where $\eta_{T,m}$ is the viscosity (in poises) of the medium at the temperature T, $\eta_{20,w}$ is the viscosity of the water at 20°C, ρ_p is the density of the particle in solution, $\rho_{T,m}$ is the density of the medium at temperature T.

Equations [1] and [2] may be combined to give:

$$s_{20,w} = \frac{(dR/dt)\,\eta_{T,m}(\rho_p - \rho_{20,w})}{w^2 R\,\eta_{20,w}(\rho_p - \rho_{T,m})} \quad (3)$$

Centrifugation in density gradients, both in swinging-bucket and zonal rotors, has been used to evaluate particle sedimentation coefficients and diameters[20,25].

To do this, we have to solve the following equation[24]:

$$s_{20,w} = \frac{\rho_p - \rho_{20,w}}{w^2 t\,\eta_{20,w}} \sum_{R_S}^{R_i} \frac{\eta_{T,mf_i}}{(\rho_p - \rho_{T,mf_i})(R_i + R_{i-1})/2} (R_i - R_{i-1}) \quad (4)$$

where i denotes a specific latex fraction.

To carry out the calculation of the sedimentation coefficient, the following parameters must be known.

- R_i, the radius from the center of the rotor to the bottom of each fraction, if the centrifuge tube is perfectly cilyndrical[20];
- R_S, the radius of the mass center of the sample zone, in this work this was monitored visually and measured with a ruler;
- w, the angular velocity in the acceleration, steady and breaking periods;
- T, temperature of the run;
- η_{T,m,f_i}, and ρ_{T,m,f_i}, refer to the average viscosity and average density of the medium in a given fraction f_i;
- ρ_p, the density of the particle in solution, experimentally determined (Figure 5);
- $\rho_{20,w}$, the density of water at 20°C;
- $\eta_{20,w}$, the viscosity of water at 20°C;
- t, may be aproximated as $(P + (A + D)/3)$[24] where A is the acceleration time, D is the deceleration time and P is the run time, in sec.

The results of the centrifugation experiment presented in Figure 7, were used to determine the sedimentation coefficients of latex particles, with the aid of the equation [4]. These calculation were easily converted to BASIC computer language and performed in an ITAUTEC 7000 microcomputer.

Based on electron microscopy literature data[26] we assumed that the latex particles are spherical, calculating their diameter according to the following equation

$$s_{20,w} = \frac{1}{18} \frac{\rho_p - \rho_{T,m,f_i}}{\eta_{T,m,f_i}} d^2 \quad (5)$$

Experimental data presented on Figure 5 with latex particles 0.305 and 0.460 μm showed that both have similar densities and therefore the mass ratio can be given by

$$\frac{m_i}{m_1} = \left[\frac{s_i}{s_1}\right]^{3/2} \quad (6)$$

where s_i and s_1 are the sedimentation coefficients in Svedberg units ($1S = 10^{-13}$ sec).

The sedimentation coefficients and the latex diameters presented in Table 2 are interpolated values calculated from the program data. The experimental values agree with nominal diameters, given by the suppliers, whithin 10% or better. Using equation [6] we calculated the mass ratios $\frac{m_i}{m_1}$ of the coagulated latex sample fractions. The results are summarized in Table 3. The coagulated sample masses fit in an arithmetic series 1,2,3,4,5,6, . . .

DISCUSSION

The procedures described in this paper are effective and can be performed in a preparative scale, at a cost much lower than those employing an ultracentrifuge. Besides that, we can collect the fractionated latex zones obtaining enough supply of material for further work. This cannot be done in the analytical ultracentrifuge. As a result, we can say that a polymer latex fractionation technique is now avaliable, which has the following properties: high resolution, ability to fractionation by size and by density/chemical composition, ability to fractionate large samples.

We should pay special attention to the faster migration rate of the osmocentrifugation cell, as compared to a glass centrifugation tube. This may be explained by considering that the moving zones introduce a local disturbance in the solvent chemical potential, even when the gradient former has reached the sedimentation equilibrium state and when a steady density gradient is reached. An osmotic solvent current should arise at the zone top and a reverse-osmotic current should occur beneath it; as a result, zone movement rate has two components: one arising from the particles themselves, moving past the surrounding liquid; another, corresponding to bulk zone motion, set on by liquid dragged by the osmotic/reverse osmotic mass current. This last point is now under detailed scruttiny, following the theoretical procedures used in former work on osmocentrifugation and will be reported elsewhere.

TABLE 2

Latex band position, sedimentation coefficients and particle diameters.

Band Radius R_i (cm)			Sedimentation[1] Coefficient (S)			Particle[2] Diameter (μm)			Nominal Diameter (μm)	Running Time (sec)
Uper	Middle	Bottom	Upper	Middle	Bottom	Upper	Middle	Bottom		
17.17	17.32	17.47			308			0.116	0.109	13331
17.57	17.65	17.72	369	417	456	0.126	0.134	0.141		
17.77	17.82	17.87	491	521	551	0.146	0.151	0.156		
19.87	20.17	20.47	1828	2033	2237	0.317	0.338	0.357	0.305	13331
23.57	23.67	23.77	4662	4757	4852	0.619	0.636	0.653		
18.97	19.22	19.47	263	297	331	0.115	0.122	0.129	0.109	62531
20.27	20.42	20.57	448	470	492	0.159	0.163	0.169		
21.07	21.22	21.37	570	593	616	0.189	0.193	0.198		
21.87	22.02	22.17	698	723	748	0.219	0.225	0.230		
22.77	22.87	22.97	850	868	886	0.253	0.258	0.263		
23.17	23.32	23.47	921	947	974	0.273	0.275	0.278		

(1) Calculated using equation [4] and using the upper, middle and bottom band coordinates.
(2) Calculated using equation [5] and using the upper, middle and bottom band coordinates.

TABLE 3

Mass ratio m_i/m_1 of the particles in the separated coagulated latex bands.

Time (sec)	Sedimentation coeffic.[1] of a coagulated latex sample fraction, s_i (Svedberg units)			Sedimentation coeffic.[1] of a latex sample, s_1 (Svedberg units)	m_i/m_1 [2]		
	Upper	Middle	Bottom		Upper	Middle	Bottom
6253i	263	297	331	297[1]	0.9	1.0	1.2
	148	170	192		1.9	2.0	2.1
	570	593	616		2.7	2.8	3.0
	698	723	748		3.6	3.8	4.0
	850	868	886		4.8	5.0	5.2
	921	947	974		5.5	5.7	5.9

(1) See Table 2.
(2) Calculated using equation [6] and using the upper, middle and bottom band coordinates.

CONCLUSIONS

Osmocentrifugation allows polymer latex fractionation size determination in short runs, in a low speed centrifuge. The fractionated zone can be collected and subjected to further analysis by conventional (IR, NMR, etc.) methods, which is not possible in analogous experiments, performed in the analytical ultracentrifuge. The movement of the sedimenting latex zone is faster in osmocentrifugation than in normal sedimentation experiment. Additionally it is evident that osmocentrifugation can be of a great help, as a gradient-forming technique for the analysis of latex particle sizes.

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