Osmocentrifugation in Density Gradients: Application to Polymer Latex Separation

ANA A. WINKLER-HECHENLEITNER, M. CÉLIA P. COSTA, and F. GALEMBECK,* *Instituto de Química, UNICAMP C.P. 6154, Campinas-SP, Brazil*

Synopsis

Polymer latexes can be fractionated by size and by chemical composition through osmocentrifugation. Density gradients are formed by centrifuging Ficoll and Percoll solutions in dialysis cells; mixed polymer latex zones are layered over these gradients and centrifuged again, leading to separated latex bands from which polymer may be collected and further analyzed. Zone migration rates in the osmocentrifugation experiment are faster than in standard centrifugation tubes.

INTRODUCTION

Polymer latexes are important components of end-use products, as well as useful intermediates in the plastics and rubber industry.[1]

Their characterization requires the evaluation of many important properties: particle size distribution and/or particle density can be determined by electron microscopy,[2] light scattering,[3] soap titration,[4] ultracentrifugation,[5] low-angle x-ray scattering,[6] flow ultramicroscopy,[7] and in Coulter counters.[8] Each of these various methods has advantages and disadvantages: ultracentrifugation, for example, is relatively fast (experiments can be completed in a few hours), absolute method; auxiliary data required are the viscosity and density of the medium and of the latex particles (which also can be determined in the ultracentrifuge). However, the required equipment is rather expensive and on-line monitoring is not feasible.

Sedimentation in density gradients has been used for density determination in isopycnic experiments, in density gradient columns, suitable for large ($\phi > 1$ mm) polymer bodies. Both isopycnic and sedimentation velocity determination in the ultracentrifuge are widely used in biological particle determination.[9]

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.[10,11] Maechtle[12] also used dynamic gradients, formed by ultracentrifugation of Percoll in H_2O, D_2O or

*Permanent address: Departamento de Química, Universidade Federal do Maranhão, 65000, São Luis-Maranhão, Brazil.

Journal of Applied Polymer Science, Vol. 34, 853–861 (1987)
© 1987 John Wiley & Sons, Inc. CCC 0021-8995/87/020853-09$04.00

MeOH. This technique was successfully used in the study of fine particles (polymer latexes, microcrystals, and bacteria cells) down to 300 nm diameter.

Recent work from this laboratory demonstrated that the osmocentrifugation technique allows blood red cell band formation in Percoll gradients much more readily than the usual centrifugation experiments.[13] In the osmocentrifugation run the solution container is a dialysis cell fitted with a highly water-permeable membrane, parallel to the centrifugation radius. Its use allows density gradient formation at low speeds; red cell bands are obtained in a single centrifugation step (instead of the usual two steps).

We have now examined the applicability of osmocentrifugation to latex particle fractionation in density gradients. The results thus obtained are described in this paper.

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. Poly(vinyl acetate) was a technical grade dispersion, from Orniex (São Paulo). Other reagents were analytical grade.

Osmocentrifugation cells[13,14] were made out of acrylic sheet or glass fiber-reinforced polyester. Typical dimensions were 7.5 × 2.3 × 0.5 cm. Cellulose acetate membranes were cast following the procedure described by Nunes et al.[15] 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 buckets and spun for the desired time until 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 heights. This is conveniently done with a long needle attached to a syringe or to tubing mounted on a peristaltic pump.

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 the cell contents are withdrawn and the densities of aliquots are determined. Representative data are shown in Figure 1 and Table I.

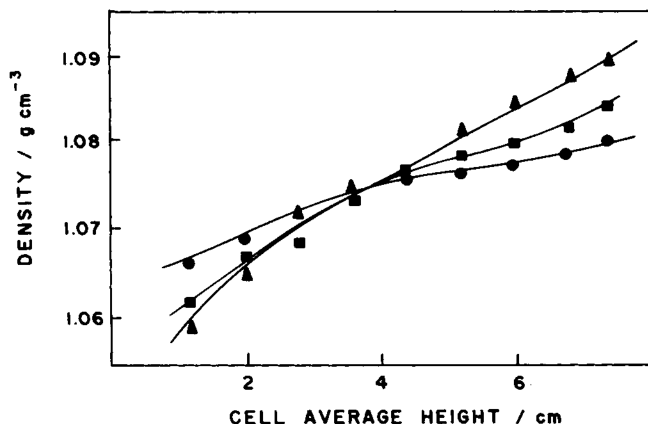


Fig. 1. Ficoll-sucrose density gradients obtained by osmocentrifugation of an 8% Ficoll, 14% sucrose solution: starting density 1.08 g/mL. Running conditions: (●) 20°C, 1 h, 1000 rpm; (▲) 20°C, 2 h, 2000 rpm; (■) 20°C, 1 h, 3000 rpm.

TABLE I
Characteristics of Density Gradients Obtained by Osmocentrifugation

Solution initial density	Running conditions t(min)/w(rpm)	Density range (g/mL)	$\Delta\rho/\Delta r \cdot 10^3$ (g/cm^4)
Percoll	15/1000	1.07–1.17	8.0
1.12 g/mL	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	60/1000	1.02–1.03	2.0
1.03 g/mL	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	70/2000	1.10–1.12	3.3
1.13 g/mL	140/2000	1.11–1.13	2.0

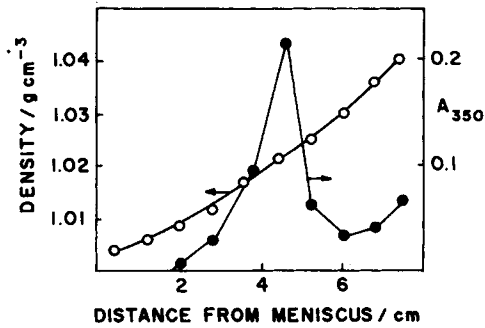
$\Delta\rho/\Delta r$: density gradient at half-height.

Polystyrene Latex Fractionation in Ficoll Density Gradients

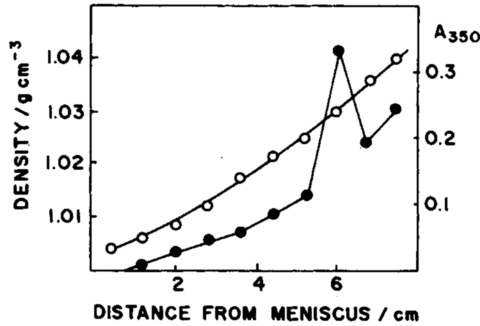
Ficoll density gradients were self-generated by osmocentrifugation of an aqueous solution (starting density 1.024 g mL^{-1}) at 3000 rpm, 20°C for 2 hours. A layer of PS latex (0.2 mL, 2% w/w) was placed over the density gradient column and the cell was spun for another 4 hours. Cell content fractions were taken at various cell heights, their refractive indexes and turbidities were measured. The results are given in Figure 2. Well-resolved latex band formation is observed both in runs performed with monodisperse latex samples and in runs performed with latex admixture.

Separation of Polystyrene-Poly(vinyl Acetate) Latexes

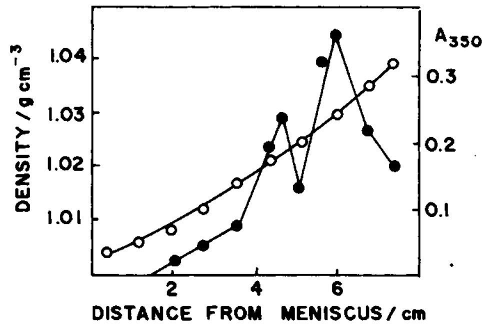
A density gradient was performed by centrifugation of Percoll (initial density 1.129 g/mL), for 50 min, 2000 rpm, 20°C in the osmocentrifugation cell: A mixture of PS (0.6%) and PVA (8%) latexes was layered on top of the



(a)



(b)



(c)

Fig. 2. 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. Sample zones were added and spun for another 4 hours.

gradient. The cell was centrifuged again, for 20 min, 2000 rpm. The formation of two bands was obtained (Fig. 3). The dispersion at each band was diluted with water, centrifuged in a glass vial (to separate the latex from Percoll); the solids collected at the bottom of the centrifugation vials were dried and extracted with toluene. The toluene-latex solutions were spread on NaCl windows and evaporated, to give thin films which were analyzed by infrared spectrophotometry. Infrared spectra thus obtained are given in Figure 4 and show that the contents of both bands are not cross-contaminated.

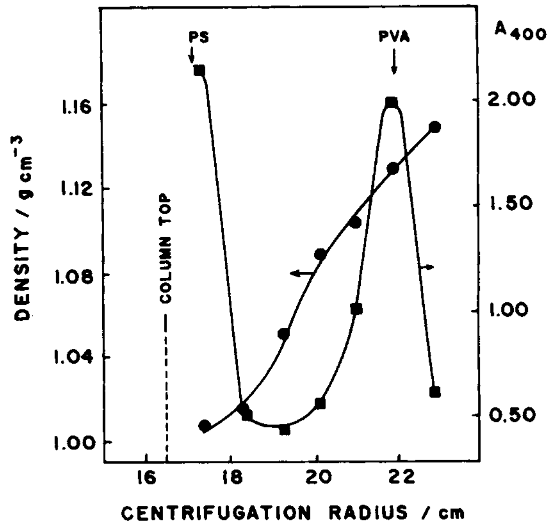
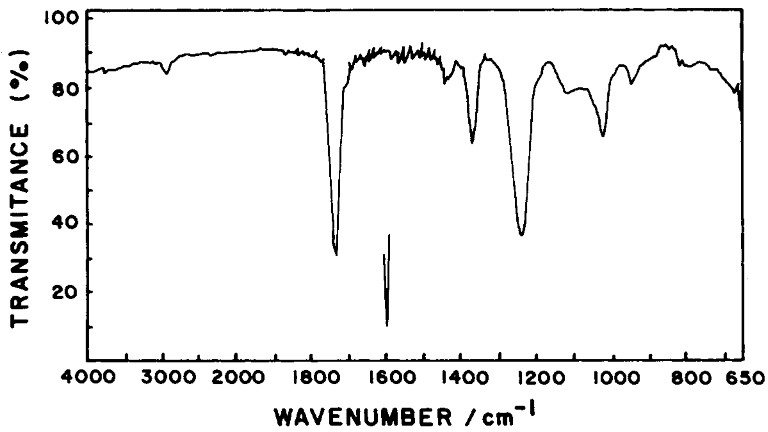
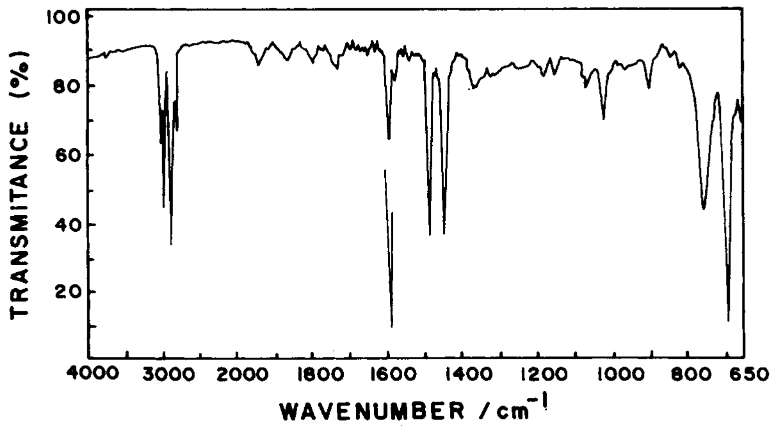


Fig. 3. PS and PVA latex particle fractionation in a preformed Percoll density gradient. Running conditions: 2000 rpm, 50 min (prior to zone layering) plus 20 min, 20°C.



(a)



(b)

Fig. 4. Infrared spectra of (a) PVA and (b) PS obtained by latex particle fractionation in a Percoll density gradient.

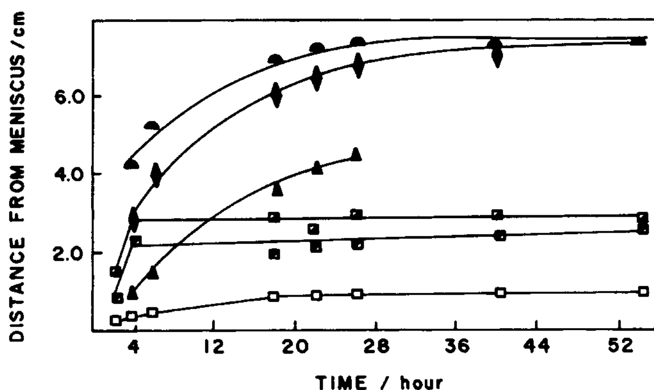


Fig. 5. PS zone and density marker displacement in the osmocentrifugation cell, as a function of time. 3000 rpm, 20°C. PS particle diameter: (●) 0.460 μm, (◆) 0.305 μm, (▲) 0.109 μm. Marker density: (□) 1.002; (◪) 1.008; (◩) 1.010 g/mL.

Density Gradient Stability in the Osmocentrifugation Cell

To determine density gradient changes in the osmocentrifugation cell, runs were performed in Ficoll gradients obtained by centrifugating 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 preformed gradient top, together with density marker beads. The cells were centrifuged (3000 rpm, 20°C); both latex band and marker bead displacement were followed. Figure 5 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 six 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 downward moving, changing density medium for the first hours and in a unchanging, immobile 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 particles). The glass tube was 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 one difference: the PS latex was layered over the gradient within the same dialysis cell in which this was obtained, and spun. The results given in Figure 6 show that isopycnic equilibrium is reached at ca. 90 hours 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 $\cdot$ cm^{-3}, in agreement with

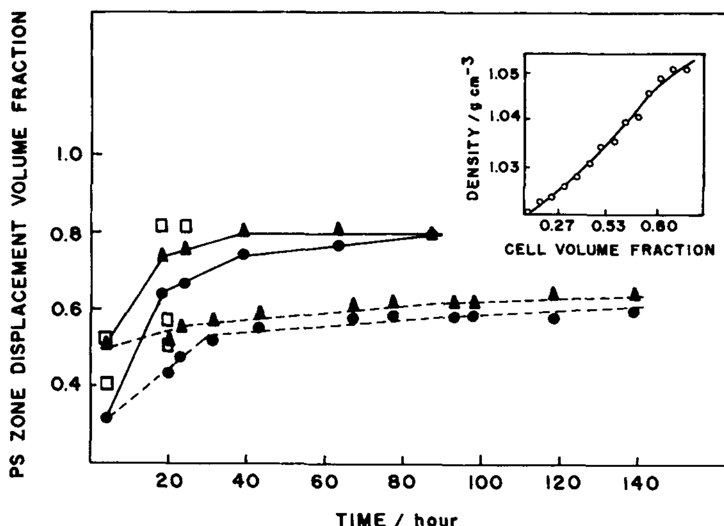


Fig. 6. PS zone displacement in the osmocentrifugation cell (—) and glass tube (---), as a function of time. Runs at 3000 rpm, 20°C. PS particle diameter: (▲) 0.460 μm, (●) 0.305 μm, (□) faint zones. The insert gives density gradient of Ficoll 8% (w/w) solution: Runs at 3000 rpm, 4 h, 20°C.

literature data.[10] 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 displacements in the osmocentrifugation cell cannot be assigned to bulk downward liquid movement. We postulate that local osmotic and reverse osmotic liquid currents are established above and below the zone, pushing it downward and faster than by independent particle movement (see Fig. 7).

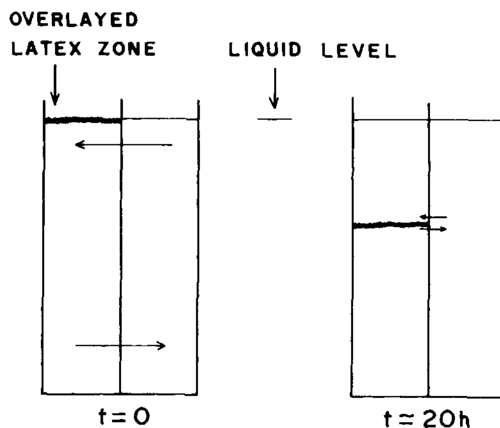


Fig. 7. Proposed mechanism for faster migration in osmocentrifugation. There is an osmotic current at the zone bottom and a reverse osmotic current at its top. The result is bulk zone (latex + solute) movement, which does not exist in normal centrifugation.

DISCUSSION

The procedures described in this paper are effective and can be performed on a preparative scale at a cost much lower than those which employ the ultracentrifuge. On the other hand, we can collect the fractionated latex zones obtaining an ample 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 available, which has the following properties: high resolution, ability to fractionate by size and by density/chemical composition, and ability to fractionate large samples.

We should pay special attention to the faster migration rate of the sedimentation cell, as compared to a glass centrifugation tube. This may be explained by considering that the moving zone introduces 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;[16] 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 currents. This last point is now under detailed scrutiny, following the theoretical procedures used in former work on osmocentrifugation[16-18] and will be reported elsewhere.

CONCLUSIONS

Osmocentrifugation allows polymer latex fractionation and size determination in short runs in a low speed centrifuge. The fractionated zone can be collected and subjected to further analysis by conventional (infrared, nuclear magnetic resonance, 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 experiments.

MCPC is a PICD-CAPES predoctoral fellow; AAWH is a CNPq predoctoral fellow. FG acknowledges support of CNPq and FAPESP.

References

1. *Encyclopedia of Polymer Science and Technology*, Interscience Publishers, New York, 1968, Vol. 8, p. 169.

2. W. N. Maclay and G. M. Gindler, *J. Colloid. Sci.*, **18**, 343 (1963).

3. I. Miroslav, *Chem. Prumysl.*, **14** (1), 19 (1964).

4. F. A. Bovey, I. M. Kolthoff, A. I. Medalia, and E. J. Meehan (Eds.), *Emulsion Polymerization*, vol. IX, in High Polymer Series, Interscience Publishers Inc., New York, 1955, p. 290.

5. H. J. Cantow, *Makromol. Chem.*, **70**, 130 (1964).

6. P. W. Kirkin and T. E. Whyte, Jr., *Am. Chem. Soc., Div. Petrol. Chem., Prepar.*, **17** (2), C32 (1972).

7. P. G. Cummins, E. J. Staples, L. G. Thompson, A. L. Smith, and L. Pope, *J. Colloid. Interf. Sci.*, **92** (1), 189 (1983).

8. M. Wales, *J. Phys. Chem.*, **66**, 1768 (1962).

9. C. A. Price, *Centrifugation in Density Gradients*, Academic Press, New York, 1982.

10. H. Lange, *Colloid Polym. Sci.*, **258** (9), 1077 (1980).

11. H. Lange, *FH-Texte*, **29**, 39 (1981).

12. W. Maechtle, *Colloid. Polym. Sci.*, **262**, 270 (1984).
13. S. P. Nunes and F. Galembeck, *Anal. Biochem.*, **146** (1), 40 (1985).
14. S. P. Nunes, A. T. N. Pires, and F. Galembeck, *Chemica Scripta*, **23** (1), 233 (1984).
15. S. P. Nunes and F. Galembeck, *J. Polym. Sci. Polym. Lett. Ed.*, **21**, 49 (1983).
16. F. Galembeck, P. R. Robilotta, E. A. Pinheiro, I. Joekes, and N. Bernardes, *J. Phys. Chem.*, **84**, 112 (1980).
17. F. Galembeck, I. Joekes, E. A. Pinheiro, J. F. Rodrigues, and N. Bernardes, *An. Acad. Brasil. Ciênc.*, **51**, 173 (1979).
18. S. P. Nunes and F. Galembeck, *J. Non-Equilibrium Thermodynamics* (in the press).

Received August 11, 1986
Accepted December 9, 1986