Velocity sedimentation of cells

T.G. Pretlow and T.P. Pretlow

Velocity sedimentation provides a variety of methods for the separation of cells according to their rates of sedimentation. Many approaches are available.

SEDIMENTATION in a continuous gradient is an ideal analytical or preparative method for the separation of molecules and particles according to their physical properties. The two most widely used forms of sedimentation are isopycnic and velocity sedimentation. Isopycnic, or density equilibrium, sedimentation is carried out with a sufficient product of force and time to permit the material being centrifuged to arrive at its intrinsic density in the gradient. Velocity sedimentation separates particles by their rates of sedimentation and is used for the characterization of macromolecules[1].

We shall address the velocity sedimentation of "cells", recognizing that what is said is also applicable to organelles, viruses, fragments of ore, and other particles. The description of sedimentation tor cells is simpler than that for molecules in that shape, degree of hydration, diffusion, and charge become negligible for these larger particles[2]. The equation for sedimentation is

$v = dr\mathrm{d}t^{-1} = a^2(D_p - D_m)\omega^2 r 18\eta^{-1}$

when v = the velocity of sedimentation, r = the distance of the cell from the center of revolution, t = duration of sedimentation, a = diameter of the cell, D_p = density of the cell, D_m = density of the medium or gradient at the location of the cell, ω = angular velocity (speed of centrifugation), and η = viscosity of the medium at the location of the cell. With small modifications for the absence of a density gradient in elutriation or for the use of unit gravity instead of a centrifugal field, this description of sedimentation is applicable to all techniques of velocity sedimentation.

Cells have two physical properties that determine simultaneously their rates of sedimentation: density and diameter. While cells can be separated according to density by isopycnic sedimentation, no method of sedimentation permits the separation of cells according to diameter. The most successful techniques for velocity sedimentation differ from older techniques in that the gradients employed have small slopes ($gml^{-1}cm^{-1}$) and are of lower density. The optimal design of such gradients has been discussed[3].

There are four commonly employed approaches to the separation of cells by velocity sedimentation: counterstreaming centrifugation or elutriation[4-6], sedimentation in a reorienting zonal rotor[7], sedimentation at unit gravity in either fixed[8,9] or reorienting[10,11] chambers, and isokinetic sedimentation[12]. The first two techniques

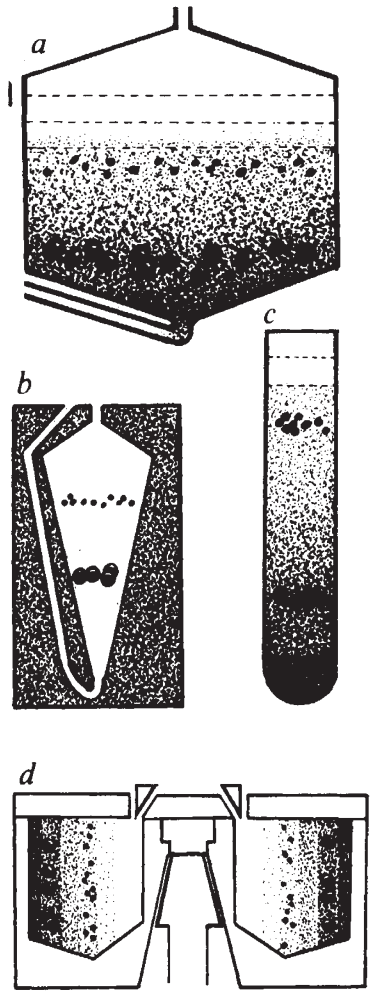

Fig. 1 Devices that are commonly used for velocity sedimentation. Density gradients are represented by variable shading. Rapidly sedimenting and slowly sedimenting cell populations are represented in the devices for comparison. *a*, Device containing a gradient for the separation of cells at unit gravity[8,9,11]. *b*, Chamber for elutriation[5,6]. *c*, Tube with 13-cm gradient for isokinetic sedimentation[2,12]. *d*, Cross-section of a reorienting zonal rotor[7].

are best suited for the separation of 10^8-10^9 cells; the last two techniques, 10^{6-10} cells. All of these techniques can be used under sterile or nearly sterile conditions. Isokinetic sedimentation and sedimentation at unit gravity are the easiest techniques to use under sterile conditions because all of the equipment required for these techniques can be autoclaved. Isokinetic sedimentation requires only equipment that is available in most laboratories, and the equipment required for sedimentation at unit gravity is inexpensive and commercially available. Both elutriation and sedimentation in a reorienting zonal rotor require particular centrifuges with expensive, specific kinds of rotors.

The most common pitfalls encountered in the use of velocity sedimentation result from a lack of familiarity with sedimentation artefacts[2]. In particular, the loss of purity that occurs when the band capacity is exceeded appears not to be widely appreciated. Other drawbacks arise from discontinuous gradients[2,13] or gradients that are suboptimally designed[3].

Improvements in velocity sedimentation are likely to occur both in the design of apparatus and in the design of specific applications. The resolution that is available with current apparatus is generally more than adequate for particles that are as heterogeneous as mammalian cells. To improve the apparatus, the steeper[3,14] centrifugal field (force per centimetre) that is obtained as the gradient is moved closer to the centre of the centrifuge may be exploited. Because the wall effect artefact increases toward the centre of the centrifuge, this approach would be more rewarding in zonal rotors and perhaps in elutriators than in parallel-walled tubes. Care must be taken not to position parallel-walled tubes too close to the centre of revolution because the wall effect becomes quite severe. Mixing more than one gradient medium to form a gradient with a viscosity which remains constant or decreases with increasing density could improve the technique. □

Thomas G. Pretlow and Theresa P. Pretlow are at the Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106, USA. For more information, fill in reader service number 100.

1. Martin, R.G. & Ames, B.N. *J. biol. Chem.* **236**, 1372–1379 (1961).
2. Pretlow, T.G., Weir, E.E. & Zettergren, J.G. *Int. Rev. exp. Path.* **14**, 91–204 (1975).
3. Pretlow, T.G. *et al. Biochem. J.* **174**, 303–307 (1978).
4. Lindahl, P.E. *Nature* **161**, 648–649 (1948).
5. Lindahl, P.E. *Biochim. biophys. Acta* **21**, 411–415 (1956).
6. Pretlow, T.G. & Pretlow, T.P. *Cell Biophys.* **1**, 195–210 (1979).
7. Pretlow, T.P. & Pretlow, T.G. in *Cell Separation: Methods and Selected Applications* Vol. 2 (eds Pretlow, T.G. & Pretlow, T.P.) 221–233 (Academic, New York, 1983).
8. Peterson, E.A. & Evans, W.H. *Nature* **214**, 824–825 (1967).
9. Hymer, W.C. & Hatfield, J.M. in *Cell Separation: Methods and Selected Applications* Vol. 3 (eds Pretlow, T.G. & Pretlow, T.P.) 163–194 (Academic, New York, 1984).
10. Bont, W.S. & Hilgers, J.H.M. *Prep. Biochem.* **7**, 45–60 (1977).
11. Wells, J.R. in *Cell Separation: Methods and Selected Applications* Vol. 1 (eds Pretlow, T.G. & Pretlow, T.P.) 169–189 (Academic, New York, 1982).
12. Pretlow, T.G. & Pretlow, T.P. in *Cell Separation: Methods and Selected Applications* Vol. 5 (eds Pretlow, T.G. & Pretlow, T.P.) 281–309 (Academic, New York, 1987).
13. de Duve, C. *J. Cell Biol.* **50**, 20d–55d (1971).
14. Pretlow, T.G. & Boone, C.W. *Science* **161**, 911–913 (1968).

© 1988 Nature Publishing Group