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Separation of different sized particles by inertial migration

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Abstract

Inertial migration has been used to fractionate feed streams containing latex particles and sheep leukocytes. For latex particles, a purified stream of small particles, less than 4 μm, was obtained. For sheep leukocytes, a stream containing almost no large leukocytes greater than 16 μm was produced. The results obtained show that this method could be used to fractionate biological cells of different sizes. Inertial migration is most suitable for the removal of larger particles which contaminate a dilute suspension of smaller particles.

Introduction

There is an ever-increasing demand for new, highly efficient separation and purification techniques in the biotechnology and biomedical industries. For example, the use of peripheral blood hematopoietic stem cells (PBHSC) in the treatment of breast carcinoma is increasing (Kessinger & Armitage 1991, Elias *et al.* 1992). However studies have shown that these PBHSC are often contaminated by breast carcinoma cells (BCC) (Moss & Ross 1992, Brugger *et al.* 1994). Consequently a number of complex methods such as flow cytometry (Gazitt *et al.* 1995) have been developed to purify PBHSC. However, these methods are very expensive and laborious. Clearly there is a demand for more efficient and cost effective separation methods.

Segré & Silberberg (1961) showed that neutrally buoyant rigid spheres suspended in a fluid flowing in Poiseuille tube flow are subject to lateral forces that translate the particle to an equilibrium radial position. This phenomenon is known as inertial migration (Bretherton 1962). Karnis *et al.* (1963) extended the initial observations of Segré & Silberberg to include non-spherical particles suspended in viscoelastic fluids. Rakow & Chappell (1987) found that *Spirulina platensis* microalgae flowing in a circular tube in laminar flow, migrated radially to a narrow annular region. In turbulent flow, however, the particles dispersed and were distributed uniformly across the tube. Rakow *et al.* (1989) and Rakow & Fernald (1991) used inertial migration to concentrate *Spirulina* particles.

The equilibrium position of a given particle in laminar tube flow depends on a number of variables including the particle and tube diameters (Cox & Brenner 1968, Ho & Leal 1974). Rakow & Fernald (1991) have shown experimentally that the equilibrium position of microalgae and plant cells (*Spirulina platensis, Chlorella vulgaris, Star astrum, Artemisia annua*) depends on the ratio of the particle to tube diameter, the Reynolds number, the axial location in the flow tube and the particle concentration.

The dependence of the equilibrium position of a given particle on the ratio of the particle to tube diameter may be used to fractionate particles of different diameters. The feed suspension, containing the particles to be separated, is introduced into the flow tube. The suspension flows through the tube in laminar flow. After the particles reach their equilibrium position, fluid from the outer region of the tube may be withdrawn using a side branch (Rakow & Fernald 1991). Particles with equilibrium positions close to the wall of the tube will be removed in the side branch

while particles with equilibrium positions closer to the centre of the tube will flow through the tube.

We have recently used inertial migration to fractionate feed streams containing a range of particle diameters. Two different feed streams were investigated, latex particles and sheep leukocytes (white blood cells). Our results suggest that inertial migration could be used to fractionate particles of different sizes in dilute solution. Here we present our results and discuss the important variables that will affect the efficiency of the separation.

Materials and methods

The flow separator

The branched flow separator used in this work is shown schematically in Figure 1. The separator consists of a glass tube, 600 μm inside diameter and 24 cm in total length. A side arm 2 mm in length and 250 μm in diameter, was located 20 cm from the feed inlet. The feed stream was pumped into the separator via a Masterflex 7565 (Cole Parmer, Vernon Hills, IL) peristaltic pump.

Particles used

Latex particles (Bangs Laboratories, Fishers, IN) of two sizes, 1 μm and 20 μm diam were mixed giving a total volume of 100 ml. A drop of Tween 20 was added in order to prevent aggregation of the particles. Units of fresh sheep whole blood were obtained from the Colorado State University Veterinary Teaching Hospital. The sheep whole blood was centrifuged at 2000 g for 15 min. After centrifugation, the plasma which has a lower density than the cells, lay above the cells. The plasma was removed and the red blood cell/plasma interface, which is rich in leukocytes, recovered. The leukocyte rich layer was then centrifuged at 2000 g for 10 min. The residual plasma was removed and the interface between residual red cells and plasma collected. Typically 2–4 ml of suspension rich in leukocytes was collected from 500 ml of sheep blood. The leukocyte rich suspension was diluted 20 times in phosphate buffered saline (PBS) (Sigma).

The particle size distribution of the feed suspensions, flow through and side branch products was measured using a Coulter LS 230 (Coulter, Miami, FL) laser diffraction particle sizer.

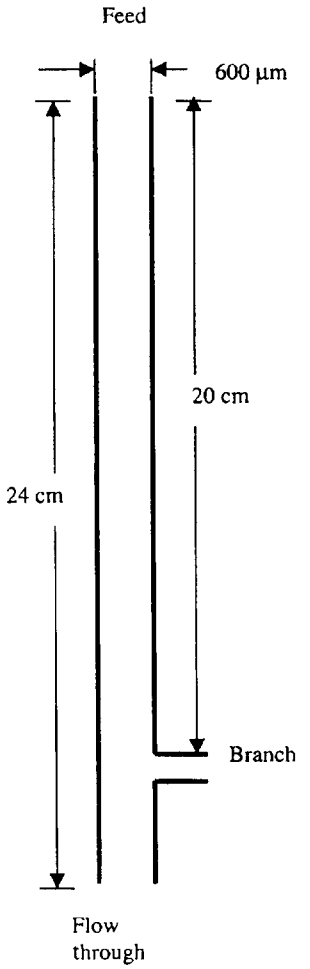

Fig. 1. Experimental set up.

Results

Figure 2 gives results for latex particles. Figure 2a shows the particle size distribution of the latex feed stream. In this figure, the percentage by volume of a given particle size is plotted against the particle size. There are two peaks, corresponding to 1 μm and 20 μm latex particles. Figure 2b compares the particle size distributions in the feed, flow through and branch streams. In these experiments the Reynolds number was 1500 (average flow velocity 2.45 ms^{-1}). As can be seen, the percentage by volume of 1 μm particles is significantly higher in the branch product compared to the flow through product.

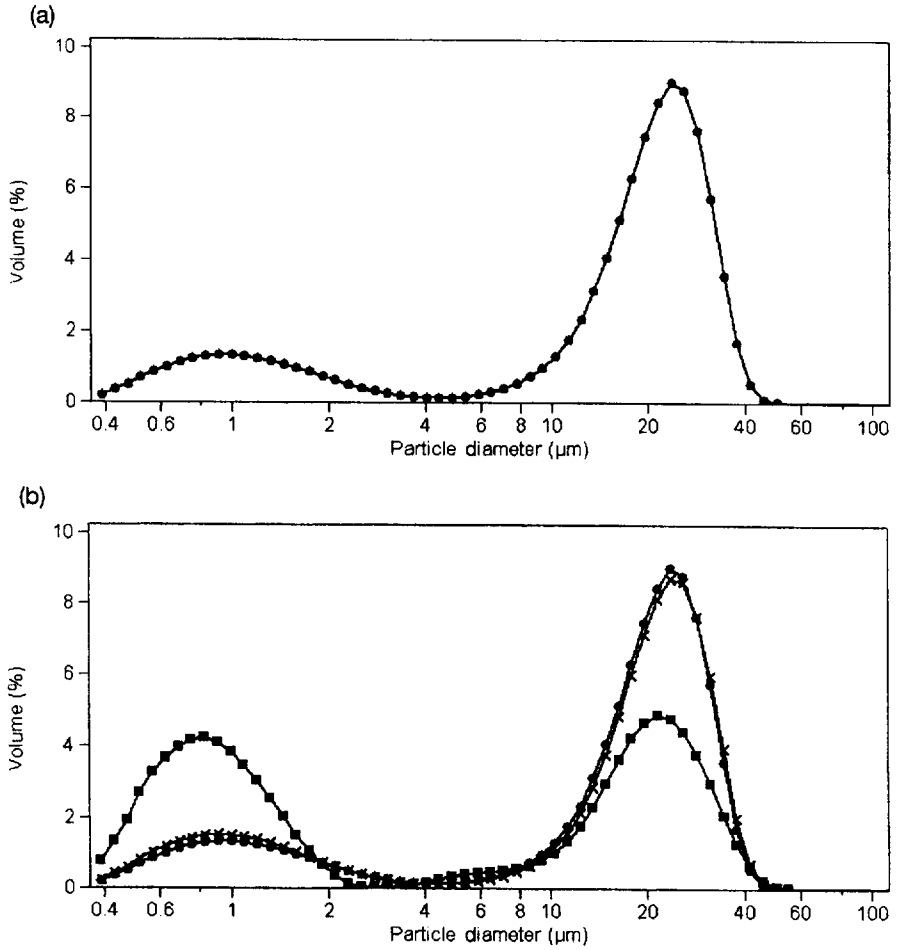

Fig. 2. (a) Latex particle feed stream. The percentage by volume of a given particle size is plotted against the particle size. (b) Fractionation of latex particles. In this figure the particle size distribution in the feed, flow through and branch streams is compared. The percentage by volume of a given particle size is plotted against the particle size. The circles, crosses and squares represent the feed, flow through and branch products respectively. As can be seen the particles size distribution in the branch product is very different to that in the feed and flow through products.

Figure 3 gives results for sheep leukocytes in which the feed stream consisted of a number of smaller cells 1.5–12 μm and a few larger cells with an average size around 15–20 μm. Table 1 gives the dimensions of sheep leukocytes. The smallest cells that are less than 6 μm are likely to be residual platelets and red blood cells. Cells in the size range 6–15 μm represent the bulk of the sheep leukocytes. A few large cells 16–20 μm are also present in the feed. These cells are likely to be large lymphocytes and monocytes. As can be seen in Figure 3b, the branch product contains very few cells larger than about 16 μm. The flow through product contains all the larger cells as well as a significant proportion of smaller cells.

Laser diffraction particle sizers determine the particle size distribution of a sample by first measuring the scattered light intensity as a function of scattering angle. Next, a distribution of spherical particles is determined that would produce the same scattering pattern. The particle sizer used here produces results that are accurate to within 1% of the mean particle size. For non-spherical particles, multimodal distributions are possible. Sheep leukocytes in suspension could form small non-spherical aggregates of two or

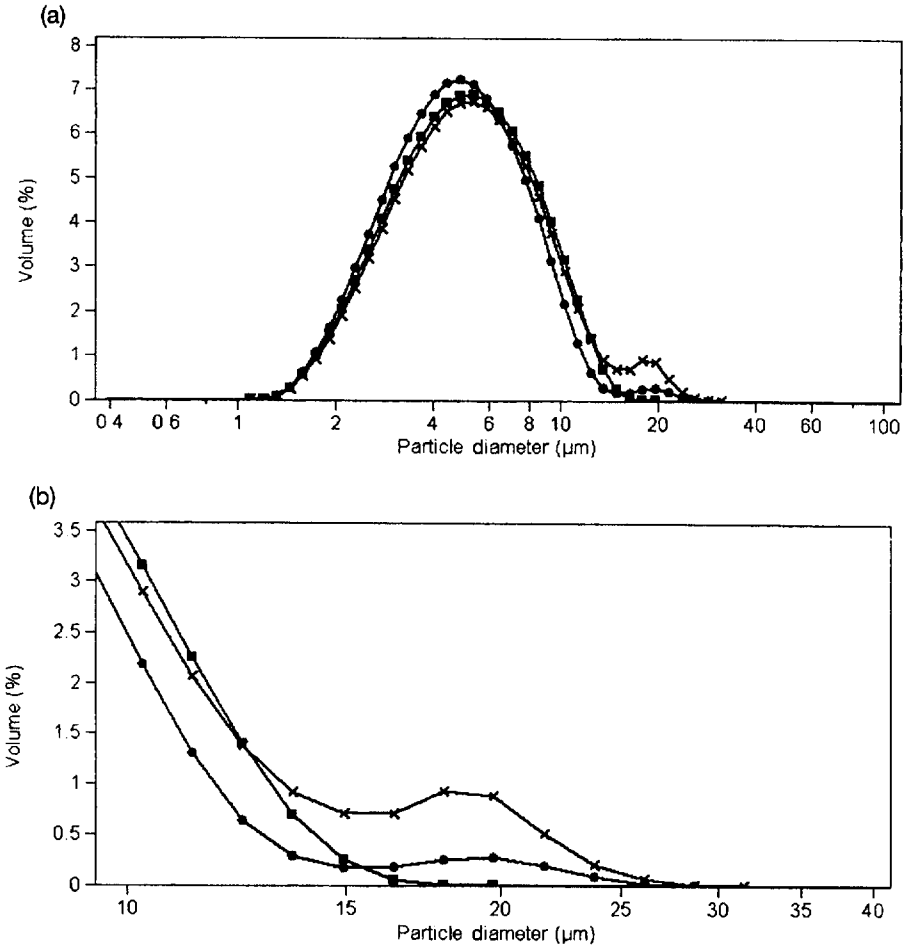

Fig. 3. (a) Fractionation of sheep leukocytes. As in Figure 2, the percentage by volume of a given particle size is plotted against the particle size. The circles, crosses and squares represent the feed, flow through and branch products respectively. No large cells are detected in the branch product. (b) Fractionation of sheep leukocytes. In this figure the peak around 18 μm in (a) is shown in more detail. As can be seen there are very few particles above 16 μm in the branch product.

three cells. Thus the particle size distributions for the feed, flow through and branch products, give qualitative information on the relative distribution of large and small cells present. The actual mean diameter of the cell could be different to that determined by the particle sizer.

Discussion

Figures 2 and 3 show that inertial migration may be used to fractionate particles. In the case of latex particles, the feed stream contained about 20% by volume particles less than 4 μm in diameter. The branch product however contained about 50% by volume particles less than 4 μm in diameter. Thus there was a significant increase in the percentage of smaller particles in the branch product. However, the flow through product had approximately the same percentage by volume particles less than 4 μm as the feed stream. This suggests that the number of particles in the branch product was much less than in the flow through product.

For sheep leukocytes the situation was different. The feed stream contained about 7% and less than 1% by volume 5 and 18 μm cells. In the branch product

Table 1. Typical size range of sheep leukocytes (Sanderson & Phillips 1981).

Blood cell	Size range (μm)
Erythrocytes	3–8
Platelets	1–3
Neutrophils	10–15
Eosinophils	10–16
Basophils	10–16
Lymphocytes	
Small	6–9
Large	12–16
Monocytes	12–18

there were very few cells above 16 μm. Unlike the latex particle feed stream, when the concentration of large particles was very low, there were almost no contaminating large particles detected in the branch product. However, as was the case for latex particles, the flow through product contained almost the same proportion of small and large cells as the feed stream. Thus, the number of cells in the branch product was again much less than in the flow through product.

These results suggest that inertial migration may be used to fractionate cells based on their size. The technique is most applicable when it is desired to purify a dilute suspension of small particles which contain a few contaminating large particles. However in order to design a viable separation process it will be necessary to optimize both the tube design and operating conditions in order to maximize the purity and recovery of the desired species and minimize the processing time. The important parameters that will affect the viability of a fractionation system are discussed below.

The higher the flow rate the lower the processing time. Thus higher Reynolds numbers will lead to shorter processing times. However in turbulent flow, all particles disperse uniformly across the tube. Thus an optimal range of Reynolds numbers is between 1200–1800. In the experiments reported here the Reynolds number was 1500.

The flow tube used (Figure 1) contained a single side branch. Poflee *et al.* (1994) describe a flow system with two side branches located 180° from each other at the same distance from the tube entrance. Using two side branches will increase the side branch flow rate and thus reduce the processing time. In our experiments the side branch was 2 mm in length. The length of the side branch relative to the remaining length of the flow tube downstream of the side branch will determine the fraction of the total flow that will pass through the side branch. The longer the side branch, the greater the pressure drop for flow through the side branch the lower the fraction of the feed stream that will flow through the side branch. This flow ratio should be carefully controlled. In order to maximize recovery of smaller particles, the side branch flow should be as large as possible without contamination by larger particles.

Determining the optimum side branch flow rate will be complex. A number of experimental studies have shown that the equilibrium radial position of a given particle depends on the Reynolds number. Rakow *et al.* (1989) and Rakow & Fernald (1991) found that the equilibrium position of helically shaped *Spirulina* moves towards the tube axis with increasing Reynolds number while that for spherical *Chlorella vulgaris* moves towards the wall. Oliver (1962) found that particles migrate closer to the axis when rotation of the particle is prevented. Further the actual equilibrium position of a given particle depends not only on the ratio of the particle to tube diameter but also on the shape of the particle.

Particle fractionation by inertial migration is only viable for particles in dilute suspension. As the particle concentration increases the particles start to interact with each other. The volume excluded by each particle, causes the zone occupied by given diameter of particles to spread. Regions occupied by particles of different diameters will start to overlap making separation difficult.

Our results show that for latex particles, while the proportion of small to large particles is significantly increased in the branch product, we still obtain contaminating large particles in the branch product. A better separation could have been obtained by reducing the ratio of branch to flow through product, thus removing less solution via the branch port. Though this would result in increased branch product purity, the recovery of smaller particles in the branch product would decrease. In the case of sheep leukocytes the ratio of small to large cells present is much higher. Thus we obtained a cleaner separation.

For both latex particles and sheep leukocytes, the recovery of the smaller particles in the branch product is very low. The recovery of smaller particles could be greatly improved by re-fractionating the flow through product either by recycling it through the original tube separator or by passing it through a second separator.

This procedure should be repeated a number of times until the desired product recovery is obtained. All the branch products should then be pooled to give a final branch product. The flow through product will eventually contain all the large particles and a few small particles.

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