

# Semi-prep Scale Separations by SPLITT

## SPLITT FEATURES

Overall, SF features simple laminar flows, perpendicularly applied forces, high separation speeds, and simple cell geometries using unpacked columns. Separation is fast because of the short separation distances, which are in the 100- $\mu\text{m}$  range. Fractionation of samples through each exit is calculable from the force equation and Stokes' law using known transport coefficients (applied forces and sample physical parameters). As with FFF based techniques,

- ❖ SPLITT allows use of solution chemistries as needed to suit the sample.
- ❖ SPLITT features low shear conditions
- ❖ Experimental conditions can be adapted to change the cut-off diameter needed -- so with two SPLITT operations a narrow size cut can be obtained.
- ❖ SPLITT is a well-characterized techniques; with information as to sample density, the user can calculate the necessary flow rates for the two inlets and outlets. Throughput calculations can be made as well as separation efficiency.
- ❖ In contrast to FFF, SPLITT can be operated in a continuous mode - gram quantities are possible.

**Example of SPLITT** Abrasive materials (boron nitride) and starch granules have both been used to demonstrate the separation and measurement of this type of particle. The following figure shows the result of setting  $d_c = 5 \mu\text{m}$  for boron nitride abrasives to remove oversized particles: Separation is fast with most run times at < 2 min. The elimination of oversized, abrasive material particles is very important in industrial polishing applications because these particles can cause scratching during surface polishing. Please note: the results below represent the type of results obtained 8 years ago, in the infancy of the technique.

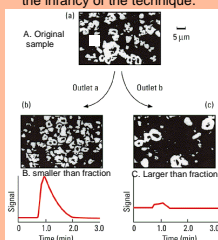


Figure 7. Separation of oversized particles ( $d_c > 5 \mu\text{m}$ ) for cubic boron nitride abrasive material. (a) Unfractionated boron nitride sample. (b) Fraction with  $d_c < 5 \mu\text{m}$  versus (c) the fraction with  $d_c > 5 \mu\text{m}$ .

The resolution of SF is proportional to the ratio between the two inlet flow rates  $[(b)/(a)]$  for samples introduced at inlet a'. Optimal inlet flow-rate ratios, which do not cause flow hydrodynamic mixing at fixed total flow rates, have been reported. (Fixed total flow rate is the sum of two inlet flow rates.) These ratios are described in the form of a universal reference plot, which shows how flow hydrodynamic mixing can be avoided in the separation process. Hydrodynamic mixing can cause separation failure.

SF throughput is directly proportional to the following variables: channel length, channel breadth, volumetric flow rate of the sample stream, and sample concentration in the feed stream. Clearly, tradeoffs between resolution and throughput are required depending on specific experimental criteria. For example, resolution requirements differ from experiment to experiment. Maintaining acceptable resolutions, which also differ from experiment to experiment, while maximizing throughputs is the best choice for preparative SF applications. For the mathematical characterization of SPLITT, please see reference list.

## Application of SPLITT to Blood and other Emulsions

Blood cell separation has been demonstrated by isolating human blood cells, platelets, and plasma proteins using the centrifugal SPLITT system. CSF successfully purified the subsets of all major white blood cells, including lymphocytes, monocytes, and neutrophils. Transport and equilibrium modes were used in series to overcome overlaps in sizes and densities of the blood cell subsets. The viabilities of the purified cells were >97%, as determined by dye exclusion testing. Throughput was  $\sim 10^{10}$  cells/h.

**Centrifugal SPLITT of Perfluorocarbon Emulsions:** Narrowing the size distributions of manufactured emulsions is very important to the pharmaceutical industry. Pharmaceutical perfluorochemical emulsions, which are used for blood substitutes, have been separated into different size distributions using centrifugal SF. The particle-size distributions, as predicted by theory, reasonably matched experimental results. Theory provided good predictions of fractionating results based on known samples and experimental parameters. Throughput was  $\sim 1.5$  g/h. Thus, SF could be a valuable technique for continuous separation of submicrometer-sized emulsions with controllable cutoff sizes

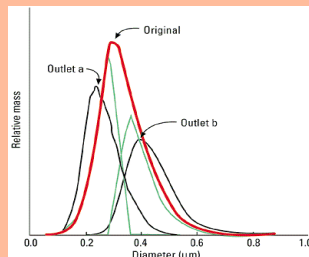


Figure 8. Size analysis of fractions obtained by CSF of a perfluorocarbon emulsion.

## Isolation of Pluripotent Cells by Mass

Recently we explored the use of CSF to isolate pluripotent, or stem, cells. This study was based on an assumption that stem cells would have a unique size. While we found CD3+ cells in different size fractions, CSF's capabilities for separation of blood cells by size was further confirmed. As a first test, we used 5 and 7 micron PS latex as a model. The following plot show a size analysis of the original, and two collected fractions.

- ❖ Purity of the "larger than fraction" was 7 micron = 94% recovery, 5 micron = 4%.
- ❖ Purity of the "smaller than fraction" was 7 micron = 6%, 5 micron = 96%

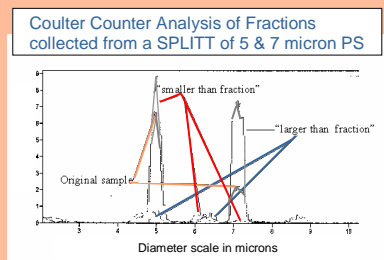


Figure 9. Analysis of the CSF test for separation of a mix of 5 & 7 micron particles

With success for the PS latex test, we continued on with a blood sample. To analyze these results, we used the Coulter Counter as well as a flow cytometer set to monitor the number of CD3+ cells. Again, size related purity was good. However, stem cells were found in both the "larger than" and "smaller than" fractions:

The original sample was characterized as have 30% small cells and 70% large, with 0.46% CD3+ cells. With respect to size,

- ❖ the smaller than fraction recovered 51% of the small and 49% of the large blood cells.
- ❖ the larger than fraction was cleaner with 92% recovery of large cells and only 8% small cells as contaminants.
- ❖ The two fractions had 0.38% and 0.68% recovery with more Cd3+ cells in larger fraction.

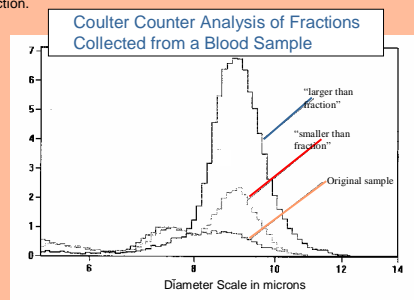


Figure 10. Size Analysis of CSF split of blood cells.