

Refractive-index-driven separation of colloidal polymer particles using optical chromatography

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Separation of equivalently sized polystyrene, $n=1.59$, poly(methylmethacrylate), $n=1.49$, and silica, $n=1.43$, beads has been accomplished using optical chromatography. The optical separations were performed using a glass flowcell that permits the optical trapping laser to be lightly focused into the fluid pathway against the fluid flow. Separation occurs due to the balance of fluid and optical forces; particles come to rest when the force due to the fluid flow equals the radiation pressure force. The ability to optically separate particles based upon their refractive index opens avenues for the characterization of colloidal samples based upon chemical characteristics, in addition to size.

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Radiation or optical pressure has been used to trap and direct microscopic particles ($0.1\ \mu\text{m}$ to $30\ \mu\text{m}$) caught in the focus of a laser beam.¹ Manipulation of the beam position, or sample container, can be used to move particles into desired positions and configurations.² The types of objects that have been optically trapped include microscopic glass and polymer spheres, viruses, bacteria, yeast, and other biological cells.^{3,4} The magnitude of the optical pressure depends on the particle size,⁵ its shape,⁶ and refractive index.⁷ Although refractive index affects the optical pressure force acting on a particle, it is a difference in chemical composition that often results in a unique refractive index. While the magnitude of optical pressure is due to several properties, laser trapping and separation research has focused primarily on the size dependence⁸ and the development of laser micromanipulation techniques.^{9–11}

In recent years, an optical separation technique has been developed, termed optical chromatography, which involves using a laser to separate colloidal particles.^{8,12,13} When particles in a liquid flowing within a capillary encounter a loosely focused laser beam propagating in the opposite direction, they are subjected to optical pressure. The optical forces draw them along the intensity gradient to the beam center (e.g., region of highest photon density). The result is that particles move against the fluid flow, along the beam center line in the direction of propagation, until the beam diverges and the photon density decreases. The particles remain stationary (become optically trapped) when the optical pressure equals the drag force exerted on the particles by the liquid flow. Optical chromatography has been applied to the separation of polymer beads and human erythrocytes based upon size,⁸ visualization of antibody–antigen reactions using labeled beads,¹⁴ determination of the optical power of motile cells and bacteria,^{15,16} and measuring the elasticity of erythrocytes.¹⁷

Harnessing the relationship between optical pressure and refractive index makes optical separation of chemically different particles possible. In this work, optical chromatography has been used for the separation of different colloidal materials. Potential separations of chemically different colloids include inorganic particles (calcium carbonate, silica,¹⁸ borosilicate, soda lime, and diamond),¹⁹ polymer particles [polystyrene (PS), poly(methylmethacrylate) (PMMA), and polytetrafluoroethylene (PTFE)], and metallic particles (nickel,¹⁸ aluminum silicate, aluminum oxide,¹⁸ and gold). With this work, the scope of optical chromatography is broadened to encompass separations based upon chemical differences in particles.

For a sphere of refractive index n_2 in a medium of lower refractive index, n_1 , the force due to optical pressure of the laser, $F_{\text{optical_pressure}}$, is given by

$$F_{\text{optical_pressure}} = \frac{2n_1P}{c} \left(\frac{a}{\omega} \right)^2 Q^*, \quad (1)$$

where P is the power of the laser, c is the speed of light, a is the sphere radius, ω is the laser beam radius, and Q^* is the conversion efficiency of optical radiation to the pressure on the particle.⁸ The term (n_1P/c) defines the incident momentum per second in a medium of refractive index n_1 .⁷ The dimensionless parameter, Q^* is obtained by integrating the function Q over the cross section of the particle in the Gaussian beam.⁸ Q defines the conversion efficiency of optical pressure transfer arising from light reflection and refraction based upon geometrical considerations.⁸ It is calculated using the Fresnel reflection and transmission coefficients, which depend upon the ratio n_2/n_1 , the refractive index ratio of the particle and the medium.

Separation in a liquid flow is measured by the distance which particles travel away from the focal point against the fluid flow. The distance traveled is the optical retention distance,⁸ z : The point at which the optical pressure equals

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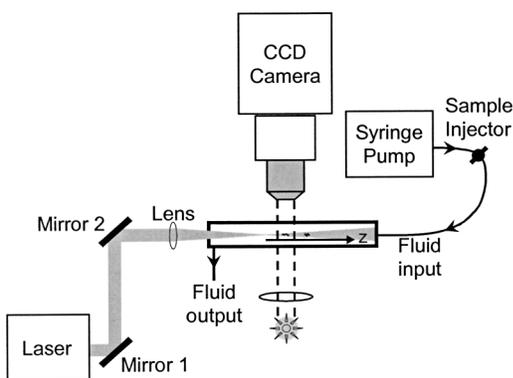


FIG. 1. Optical separation instrument with laser focused into the flowcell, opposite to the direction of fluid flow. The retention distance, z , direction is denoted inside the flowcell.

the force exerted on the spheres by the liquid molecules and is defined, according to

$$z = \frac{\pi \omega_0^2}{\lambda} \sqrt{\frac{n_1 P Q a}{3 \pi \eta \nu c \omega_0^2} - 1}, \quad (2)$$

where the power of the TEM₀₀ mode laser is P , c is the speed of light, a is the sphere radius, ω_0 is the beam radius at the focal point, λ is the wavelength of light, ν is the velocity of the particle in the liquid flow, and η is the viscosity of water. The refractive index of the particle is used in the calculation of the efficiency of optical pressure transfer, Q . A more detailed description of the theory can be found elsewhere.⁸

The optical chromatography system consisted of an argon-ion laser (Innova 100, Coherent Laser, Inc.) operating at 488 nm with a power of 0.75–1.0 W. An illustration of the system is given in Fig. 1. The beam was focused into the flowcell (described below) using a 1 in. diameter, 50 mm focal length planoconvex focusing lens. The flow cell was mounted on a five-axis positioner (New Focus, San Jose, CA), which allowed precise alignment of the beam within the flowcell. Images were collected using a microscope (Leica DMRX) with a cooled charge-coupled device camera. Low magnification, typically 25×–50×, was required to be able to visualize the large separation distances in one image. Due to the low magnification, images of the particles were most easily visualized in laser light scatter. This was accomplished using a 488 nm partially transmitting filter (Epi-fluorescence filter module, Chroma Inc.). The flow system consisted of a 10 mL syringe and a syringe pump (PHD2000, Harvard Apparatus) connected to 0.02 in. inner diameter PTFE tubing. Bead samples were introduced into the flow via an injector (Rheodyne), fitted with a 2 μ L injection loop. The glass flowcell used for separations was assembled using four rectangular glass microscope slides and a fused silica window with UV-cured epoxy. The slides were arranged to form an “L”-shaped channel. The fused silica window was epoxied to the open end of the fluid path, thus sealing it and providing clear optical access to the channel. The dimensions were 1 mm high, 1 mm wide, and 75 mm long resulting in a volume of 75 μ L.

Chemically different but uniformly sized spheres were obtained from two sources: 2.25 μ m \pm 0.11 μ m diameter PS and 2.20 μ m \pm 0.11 μ m diameter PMMA beads (Magsphere, Inc., Pasadena, CA), and 2.30 μ m \pm 0.10 μ m diameter silica



FIG. 2. Optical separation of uniformly sized PS and PMMA, shown in laser light scatter. The laser ($P=0.88$ W) was propagating from the left- to the right-hand side and the flow (fluid linear velocity=51 μ m/s) traveling from the right- to the left-hand side.

particles (Bangs Laboratories, Inc., Fishers, Ind.). The 0.1 μ m size standard deviation theoretically would result in only a 60 μ m difference in optical retention distance, which is negligible compared with the >600 μ m separations observed in these experiments. All samples were dispersed in water with 2% sodium dodecyl sulfate (Sigma-Aldrich Corp., St. Louis, MO) added to minimize aggregation. The refractive indices of the beads, as reported by the manufacturer, are PS, $n_2=1.59$, PMMA, $n_2=1.49$, and silica, $n_2=1.43$.

A diluted binary mixture of the uniformly sized PS and PMMA beads was injected into the flowcell and an optical separation resulted, as seen in Fig. 2. The laser propagates from left- to right-hand side and the fluid flow travels from the right to the left-hand side. The average retention distance of the downstream group of beads (on the left-hand side of image) was 1.33 mm \pm 0.04 mm which compares reasonably well with the theoretically predicted retention distance of PMMA beads, 1.57 mm. For the upstream group of beads (on the right-hand side of image), the average retention distance was 2.15 mm \pm 0.03 mm which compares well with the theoretically predicted retention separation distance of PS beads, 2.35 mm. Another PS and PMMA separation was performed and the linear velocity then varied to further demonstrate the stability of the separation and the agreement with theory. The retention distances of the PS and PMMA particles as functions of several different linear velocities are shown in Fig. 3. The theoretical model (smooth line) is based upon Eq. (2)

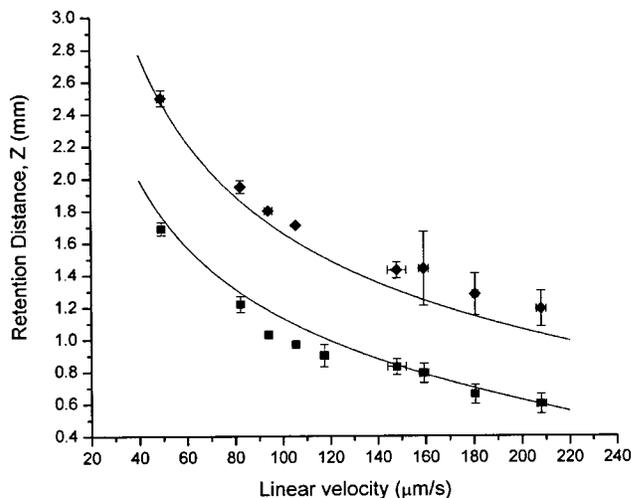


FIG. 3. Retention distance as a function of linear velocity for uniformly sized PS (diamonds) and PMMA (squares) beads. The smooth curves are the theoretically predicted retention distances for PS and PMMA. The laser power was constant at 0.96 W.

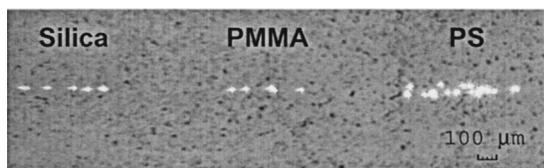


FIG. 4. Three component optical separation of uniformly sized PS, PMMA, and silica beads shown in laser light scatter. Experimental conditions: Laser power=0.77 W and fluid linear velocity=32 $\mu\text{m/s}$.

and known optical, fluidic, and colloidal parameters of the experiment. Both PS and PMMA particles experience a rapid drop in the retention distance from linear velocities of 50 $\mu\text{m/s}$ to 120 $\mu\text{m/s}$ and then less retention distance decrease up to 210 $\mu\text{m/s}$. The PS and PMMA particles were completely separated over the entire linear velocity range, and the agreement with theory is reasonably good: The percent errors between theoretical and experimental observations were between -9% and 16% . Finally, a separation of a three component mixture of the uniformly sized PS, PMMA, and silica beads was attempted and the results can be seen in Fig. 4. All three types of beads, with different refractive indices but uniform sizes were well separated in the laser beam.

The separation of colloidal suspensions based upon differences only in refractive index has been demonstrated using optical chromatography. Three different types of beads, PS ($n_2=1.59$), PMMA ($n_2=1.49$), and silica ($n_2=1.43$) beads have been optically separated. The potential for optical separation or fractionation of colloidal samples of different chemical composition is great. In particular, biological specimens of similar sizes would benefit from optical separation capabilities, based upon refractive index differences.²⁰ Given the chemical sensitivity of the demonstrated method, its future application to areas of biochemistry, molecular biology,

and microbiology offers significant potential for advanced analysis, characterization, and separation.

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- ¹A. Ashkin, *Phys. Rev. Lett.* **24**, 156 (1970).
- ²M. M. Koshioka, K. Sasaki, N. Kitamura, and H. Masuhara, *Opt. Lett.* **16**, 19, 1463 (1991).
- ³A. Ashkin, J. M. Dziedzic, and T. Yamane, *Nature (London)* **330**, 769 (1987).
- ⁴A. Ashkin and J. M. Dziedzic, *Science* **245**, 1517 (1987).
- ⁵A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, *Opt. Lett.* **11**, 5, 288 (1986).
- ⁶R. C. Gauthier, M. Ashman, and C. P. Grover, *Appl. Opt.* **38**, 22, 4861 (1999).
- ⁷A. Ashkin, *Biophys. J.* **61**, 569 (1992).
- ⁸T. Kaneta, Y. Ishidzu, N. Mishima, and T. Imasaka, *Anal. Chem.* **69**, 2701 (1997).
- ⁹K. Taguchi, H. Ueno, Y. Hiramatsu, and M. Ikeda, *Electron. Lett.* **33**, 5 (1997).
- ¹⁰C. Mio, T. Gong, A. Terray, and D. W. M. Marr, *Rev. Sci. Instrum.* **71**, 5, 2196 (2000).
- ¹¹J. E. Curtis, B. A. Koss, and D. G. Grier, *Opt. Commun.* **207**, 169 (2002).
- ¹²T. Imasaka, Y. Kawabata, T. Kaneta, and Y. Ishidzu, *Anal. Chem.* **67**, 1763 (1995).
- ¹³J. Makihara, T. Kaneta, and T. Imasaka, *Talanta* **48**, 551 (1999).
- ¹⁴S. Miki, T. Kaneta, and T. Imasaka, *Anal. Chim. Acta* **404**, 1 (2000).
- ¹⁵N. Mishima, T. Kaneta, and T. Imasaka, *Anal. Chem.* **70**, 3513 (1998).
- ¹⁶T. Kaneta, N. Mishima, and T. Imasaka, *Anal. Chem.* **72**, 2414 (2000).
- ¹⁷T. Kaneta, J. Makihara, and T. Imasaka, *Anal. Chem.* **73**, 5791 (2001).
- ¹⁸S. Koyanaka and S. Endoh, *Powder Technol.* **116**, 13 (2001).
- ¹⁹H.-K. Chung and J. C. Sung, *Diamond Relat. Mater.* **10**, 1584 (2001).
- ²⁰J. B. Bateman, J. Wagman, and E. L. Carstensen, *Kolloid Z. Z. Polym.* **208**, 44 (1966).