

Note: High-precision microsphere sorting using velocity sedimentationDaniel Cheng,¹ Ken Halvorsen,² and Wesley P. Wong^{2,a)}¹*Brown University, Providence, Rhode Island 02912, USA*²*Rowland Institute at Harvard, Harvard University, Cambridge, Massachusetts 02142, USA*

(Received 26 October 2009; accepted 11 January 2010; published online 12 February 2010)

Monodisperse populations of microspheres are desirable for a variety of research and industrial applications, but many desirable sizes and materials can be difficult to synthesize and have limited commercial availability. In this paper, we present an effective, straightforward, and low cost method for sorting polydisperse microspheres into many separate monodisperse samples. The basic approach is to use velocity sedimentation through a density gradient in a long vertical column, followed by carefully targeted extraction. We demonstrate this technique by reducing the coefficient of variation of melamine microspheres from 13% to 1%–4% and glass microspheres from 35% to 3%–8%. This simple and inexpensive method can be used to sort microspheres of many sizes and materials, and is easily scalable, opening the possibility of cheap, monodisperse microspheres. © 2010 American Institute of Physics. [doi:10.1063/1.3302828]

Microspheres and nanospheres of various materials and sizes are used in a wide range of industrial and research applications. Industrially, they found uses in many consumer products¹ such as paints, ceramics, cosmetics, and electronics. In research, their uses include molecular purification,¹ drug delivery,² imaging,^{3,4} photonics,^{5,6} and single-molecule force studies.⁷ For many applications it is critical or at least desirable to have a monodisperse population. However, highly monodisperse bead samples can be difficult or impossible to produce with conventional methods depending on the material and size range. In certain cases where monodisperse beads are available (e.g., “size standards”), they can be dramatically more expensive.

There has been some significant effort to produce monodisperse beads by improving on or designing new methods of synthesis.¹ Coefficients of variation in commercially available microspheres range widely from less than 1% to greater than 30%, depending on the size and material. Materials such as polystyrene and silica are available with low CVs (1%–3%) in certain size ranges, typically at an additional cost. However, for some materials such as borosilicate glass there are no commercially available monodisperse beads (e.g., 35% for 2 μm size-standard beads). Considering these challenges in monodisperse microsphere production, it is surprising that little work has been done on sorting polydisperse beads into monodisperse samples. Previous sorting methods have not been shown to improve the CV of commercial beads.^{8–11}

In this paper we present a simple and effective method for sorting microspheres into many monodisperse samples. We employed gravity driven velocity sedimentation through a density gradient to separate beads of different sizes, similar to cell sorting techniques.¹² Similar methods using density gradient centrifugation have recently been used to sort clusters of microspheres.^{13,14} When placed in a vertical

column of liquid, isolated microspheres and nanospheres will experience a gravitational force, a buoyant force, and a drag force. The downward force is simply $F_{\text{down}} = 4\pi(\rho_p - \rho_f)gR^3/3$, where g is acceleration due to gravity, R is the particle’s radius, and ρ_p and ρ_f are the mass densities of the particle and the fluid, respectively. The drag force will be proportional to the particle’s velocity as described by Stokes’ law: $F_{\text{drag}} = 6\pi\mu RV$, where μ is the fluid’s dynamic viscosity and V is the particle’s velocity. The settling velocity is then given by

$$V_s = \frac{2(\rho_p - \rho_f)}{9\mu}gR^2. \quad (1)$$

Therefore, beads of the same material but different sizes will travel at different velocities through the column, separating throughout the length of the column. Fluid volumes extracted at different heights will contain beads of different average size, with each sample being more monodisperse than the initial bead suspension. Precision can be made higher with longer columns or possibly with smaller extraction volumes, given that no fluid disturbances are introduced.

The column used for sedimentation was custom built from a 4 ft long polycarbonate tube with a 1 in. OD and 3/4 in. ID. 17 KYNAR (polyvinylidene fluoride) ports are attached with 3/32 in. ID to a 10–32 UNF barbed fitting, each 1 cm apart starting from the base of the column. Each of these ports is attached to a short length of Tygon R-3603 tubing and is stopped using a ratchet clamp. 4 cm above the top small port is a polyurethane port with 1/4 in. ID to a 1/4–28 UNF barbed fitting. The larger polyurethane ports then continue at 8 cm intervals up to the top of the column. These are initially stopped using PTFE tape. The material cost of the apparatus was under \$100, and only simple machining was required to attach the fittings.

A sucrose gradient was used in the sedimentation column to stabilize the particles as they traveled down the column. This reduces convection effects in the fluid and the Rayleigh–Taylor instability,¹⁵ which causes pluming of the microspheres as they travel down the column. The use of

^{a)} Author to whom correspondence should be addressed. Electronic mail: wong@rowland.harvard.edu.

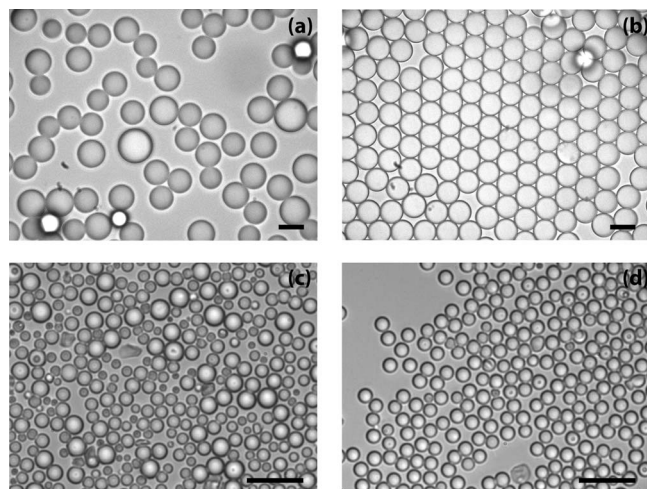


FIG. 1. Images of (a) stock 10 μm melamine microspheres, (b) high-precision sorted melamine microspheres, (c) stock 2 μm glass “size standard” microspheres, and (d) sorted glass microspheres. Scale bars are 10 μm .

density gradients is an established method in biology^{12,16} but has only recently been used for sorting colloids via centrifugation.^{13,14} The gradient was formed sequentially with a 50 ml gradient former (Jule Biotechnologies) with the highest mass percent solution at the bottom.

Careful bead deposition at the top of the column with minimal fluid disturbance is of critical importance. Beads suspended in ethanol were trickled down the side of the column using a 2 ml serological pipet (VWR), where they initially floated above the sucrose solution due to the density mismatch. Before deposition and ethanol suspension, the beads were vortexed and sonicated to thoroughly mix the different sizes of beads and to ensure that none were bound together. Beads were processed in batches of 200 μl of 0.5% solids.

The beads were recovered by sequentially opening and draining each port from the top down. The top ports were punctured using a syringe and drained in a waste beaker, while the bottom ports were drained into individually labeled vials. This differs from standard cell sorting techniques, in which sorted cells are typically retrieved by draining fluid from the bottom of the column.¹² Finally, the beads were concentrated as needed using centrifugation.

The microspheres used for testing of the bead sorting apparatus were 10 μm diameter melamine formaldehyde microspheres (Corpuscular) and 2 μm certified size standard borosilicate glass beads (Duke Scientific). Melamine beads were run through 83 cm of liquid (30%–5% sucrose gradient) and extracted after 26 h, while glass beads were run through 53 cm of liquid (15%–5% sucrose gradient) and extracted after 28 h.

Measurement of the bead sizes was accomplished with video microscopy image analysis. We used an optical microscope with a CCD camera and processed images with ImageJ using the analyze particles tool. All measurements of bead size and CV were made in this way unless otherwise noted.

In both trials with melamine and glass microspheres, our method was successful in dramatically reducing the size distribution (Fig. 1). The melamine beads were sorted with a

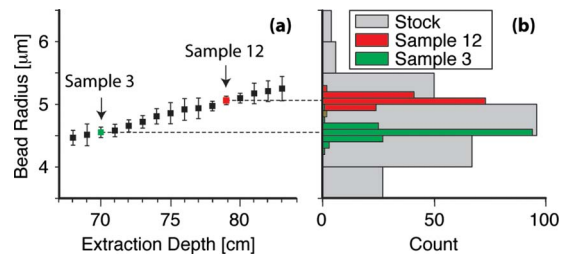


FIG. 2. (Color online) (a) Mean radii of sorted melamine microspheres as a function of distance traveled at 26 h, with vertical bars depicting the standard deviation of each sample. (b) Histograms of radii for the stock melamine microspheres (grey) and two sorted samples (red and green on-line, darker grey in print).

long (83 cm) column, leading to high precision. In fact, some sorted melamine samples were so uniform that they formed nearly perfect crystalline structures when close packed, despite originating from a polydisperse sample. In contrast, the glass beads were sorted with a shorter (53 cm) column leading to quicker sorting but slightly lower precision.

For the melamine beads, the average coefficient of variation (CV) was reduced from the initial 13% to less than 3% with a range of 1%–4%. Results from the glass beads were even more dramatic since the starting CV was 35% (as quoted by the manufacturer). Even sorting with the shorter column produced up to a tenfold reduction in polydispersity, with sample CVs ranging from 3%–8%, averaging just under 5%.

As expected, mean microsphere size depended on the location of the extraction volume, with larger microspheres being further down the column [Fig. 2(a)]. For the melamine beads, each additional 1 cm in distance traveled selected for microspheres on average 50 nm larger than the previous. Compared to the original size distribution, the sorted beads were remarkably uniform [Fig. 2(b)].

We successfully developed and employed a new sorting method that dramatically decreases size variations in microspheres. Our results indicate that CVs lower than those available commercially for many bead sizes and materials are readily achievable. Demonstrated for two bead types in this paper, our method can be applied to other sizes and materials. Furthermore, it may be possible to improve the precision of sorting by using longer columns or smaller extraction volumes.

When applying this method to a new bead size or material, it is important to consider the required column length and extraction volumes necessary to achieve a desired CV in bead radius. As shown in Eq. (1), the bead velocity, and thus the distance traveled at a given time, scales with R^2 . Consequently, the distance between extraction sites as a percentage of total column length should be roughly twice the desired CV percentage. For example, a desired CV of 1% would require the distance between extraction sites to be 2% of the total column length. A 1 m tube with 2 cm spacing would satisfy this, as would a 10 cm tube with 2 mm spacing. This rough estimate is for the idealized scenario where no fluid is disturbed, all beads begin at the same height, and Stokes’ law applies. Applied to our experiments, we would have predicted CVs of 0.6% and 0.9% for melamine and glass beads, respectively. However, our measured CVs were higher than this, presumably due to fluid disturbances upon deposition or

extraction, and spread in the initial bead distribution. These factors may also account for the variation in CV from sample to sample. While negligible in our examples, for particularly small beads or short columns, diffusion may also need to be taken into account.

Once the column height has been specified, sedimentation time can be coarsely estimated using Eq. (1) (i.e., by integrating $1/V_s$ over the height of the column) or calibrated directly with a practice run. For our calibration runs, we ran a visible quantity of beads (dyed, or in high concentration) through the column and recorded the time.

Our high-precision sorting method is passive, inexpensive, and easily scalable to larger volumes by using a larger diameter column or multiple columns. This opens up the possibility of sorting beads on an industrial scale, perhaps as a substitute for synthesizing monodisperse microspheres. In certain cases it may be more efficient or cost effective to produce highly polydisperse beads and use our method to sort them as a postproduction step. Alternatively, end users can implement our method for more direct control over the precision of the microspheres they buy.

The authors would like to thank Diane Schaak, Howard Berg, Vinothan Manoharan, and Linda Stern for insightful

discussions. We would like to thank Don Rogers for machine shop help.

- ¹H. Kawaguchi, *Prog. Polym. Sci.* **25**, 1171 (2000).
- ²S. Freiberg and X. X. Zhu, *Int. J. Pharm.* **282**, 1 (2004).
- ³A. L. Klibanov, *Adv. Drug Delivery Rev.* **37**, 139 (1999).
- ⁴H. F. Zhang, K. Maslov, G. Stoica, and L. V. Wang, *Nat. Biotechnol.* **24**, 848 (2006).
- ⁵T. Yamasaki and T. Tsutsui, *Appl. Phys. Lett.* **72**, 1957 (1998).
- ⁶F. Vollmer, S. Arnold, D. Braun, I. Teraoka, and A. Libchaber, *Biophys. J.* **85**, 1974 (2003).
- ⁷K. C. Neuman and A. Nagy, *Nat. Methods* **5**, 491 (2008).
- ⁸S.-K. Hoi, C. Udalgama, C. H. Sow, F. Watt, and A. A. Bettiol, *Appl. Phys. B* **97**, 859 (2009).
- ⁹G. Milne, D. Rhodes, M. MacDonald, and K. Dholakia, *Opt. Lett.* **32**, 1144 (2007).
- ¹⁰F. C. Cheong, C. H. Sow, A. T. S. Wee, P. Shao, A. A. Bettiol, J. A. van Kan, and F. Watt, *Appl. Phys. B: Lasers Opt.* **83**, 121 (2006).
- ¹¹M. Yamada, M. Nakashima, and M. Seki, *Anal. Chem.* **76**, 5465 (2004).
- ¹²R. G. Miller and R. A. Phillips, *J. Cell. Physiol.* **73**, 191 (1969).
- ¹³V. N. Manoharan, M. T. Elsesser, and D. J. Pine, *Science* **301**, 483 (2003).
- ¹⁴P. Johnson, C. M. van Kats, and A. van Blaaderen, *Langmuir* **21**, 11510 (2005).
- ¹⁵D. H. Sharp, *Physica D* **12**, 3 (1984).
- ¹⁶R. Hinton, *Density Gradient Centrifugation* (Elsevier, North Holland, 1976).