Density-Based Diamagnetic Separation: Devices for Detecting Binding Events and for Collecting Unlabeled Diamagnetic Particles in Paramagnetic Solutions

SUPPORTING INFORMATION

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EXPERIMENTAL SECTION

Devices for Magnetic Separations: Two NdFeB magnets (2 in. × 2 in. × 1 in., Edmund Scientific Co.) were held 1.75-in. apart with like poles facing each other. The magnets were held in place and aligned by two aluminum blocks (6-in. × 4-in. × 2-in. with a 2-in. × 2-in. × 1-in. cavity in the center to host the magnets) that were brought together by a 3-kg weight placed on the upper magnet and connected by three brass, threaded runners with nuts as stoppers.

Separation of Particles in Aqueous Solutions: Precision density standard beads (American Density Materials, Inc.) with densities ranging from 1.1000-1.8000 (± 0.0001 g/cm^3) were used to calibrate the system. Aqueous solutions of $GdCl_3$ (Aldrich) with concentrations between 0.1-1.9 M were prepared in 4-mL glass vials. The density standard beads were placed in each of the different Gd^{3+} solutions and the height at which the center of the bead rested was recorded when the vial was aligned to the axis of the magnets. A height of 0.0 mm was recorded for spheres that rested on the bottom of the vial.

To optimize the separations, the Gd^{3+} solution chosen should be slightly less dense than the least dense particle in the mixture. A more concentrated solution would cause some material to float and thus complicate the separation. A less concentrated solution would lead to weaker magnetic forces, thus yielding a poorer separation, i.e., smaller distance between the separated bands of beads.

Separation of Particles in Non-Aqueous Solutions: Solutions of 360-mM and 435-mM $GdCl_3$ in methanol were prepared. One piece of polystyrene (M_w ~ 280,000; Aldrich), isotactic polypropylene (M_w ~ 340,000; Aldrich), and polyethylene (M_w ~ 15,000; Aldrich) were placed in each solution in a 4-mL glass vial that was aligned with the axis of the magnets for those experiments carried out under an applied magnetic field.

Detection of Protein Binding to a Solid-supported Small Molecule: To a glass solid-phase reaction vessel was added 300 mg of acryloylated O,O′-bis(2-aminopropyl)polyethylene glycol/dimethylacrylamide copolymer (PL-PEGA-NH_2) (Polymer Laboratories, 0.2 mmol amine/g polymer, 300-500 μM), biotin (Sigma, 0.073 g, 299 μmol), O-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (Aldrich, 114 mg, 299 μmol), 1-hydroxy-7-azabenzotriazole (Aldrich, 0.046 g, 299 μmol), and diisopropylethylamine (Aldrich, 0.052 mL, 299 μmol). The entire mixture was diluted in 3 mL of anhydrous dimethylformamide (DMF) (Aldrich) and the vessel was agitated using a wrist-action shaker (Burrell) for 18 h. The polymer beads were washed with DMF (3 × 3 mL), methylene chloride (3 × 3 mL), 95% ethanol (2 × 3 mL), and Millipore-purified water (2 × 3 mL); the beads were agitated using the wrist-action shaker for 30 s between washes and the solvents were removed using a positive pressure of nitrogen.

Approximately 20 beads of this biotin-labeled material were transferred to a 1.5-mL cuvette. These beads were soaked for 15 min with 21.2 μL streptavidin (PIERCE, immunoPure; 189 μM in 40 mM potassium phosphate buffer, pH 7.0). The beads were diluted with 1.5 mL of a pH 7.3, 135 mM diethylenetriaminepentaacetic acid Gd(III) salt solution containing 40 mM potassium phosphate buffer (pH 7.0) and 0.05% TWEEN 20 (Mallickrodt); before use, this Gd^{3+} solution was filtered twice through a 0.2 μM PVDF membrane (PALL Life Sciences). Another 20 beads of the pure biotin-labeled material were added to this Gd^{3+} solution and the entire mixture was agitated with a spatula by gently plunging the spatula in the solution five times. The cuvette was placed in the center axis of the magnets and the beads reached an equilibrium levitation height in 30 min.

Electrostatic Adhesion of Gold Nanoparticles to Chemically Modified Microspheres: Polystyrene microspheres (50-μm diameter, Duke Scientific) were chemically modified to yield microspheres with tetraalkylammonium chloride functionality.[40] Gold nanoparticles (~12-nm diameter) were synthesized with a citrate capping layer using the process reported by Shipway *et al*.[45] Sets of positively modified and unmodified Merrifield resin spheres were placed together in a solution containing 500 μL of water, 500 μL of DMF, 100 μL of the previously described gold nanoparticles (0.01% w/v in water), and 25 μL of surfactant (TWEEN 20). After 1 hour of shaking the microspheres, we filtered, collected and re-suspended them in an aqueous, 300 mM solution of $GdCl_3$. Finally, we placed the solution with beads in a cuvette that was aligned with the axis of the magnets.

Design of the Microfluidic System:

The Injection System. A 60-mL polyethylene syringe was filled with the samples to be separated in a 250 mM $GdCl_3$ aqueous solution. The syringe was held vertically (perpendicular to the benchtop) in a syringe pump (Harvard Apparatus PHD 2000) and connected using a three-way valve to a 16-gauge needle (Becton Dickinson & Co.). The three-way valve permitted the refilling of the syringe by sealing off the tubing. The needle was connected to the PDMS separation device with a minimal length of polyethylene tubing (PE 205, I.D. 1.57 mm, O.D 2.08 mm, Becton Dickinson & Co.), also held vertically.

The Separation Device. We fabricated a thick master for the separation device using a UV curable epoxy (NOA81, Norland Products Inc.) by exposing it to non-collimated UV light (long-wave mercury lamp, 100 W) through a transparency photomask.[46] The thickness of the master was ~2 mm; thick enough such that the outer diameter of the inlet/outlet tubing could be incorporated into the device. This thickness permitted particles as large as ~500 μm in diameter

to flow easily through the system without adhering to the walls or clogging the channel. The separation device was made by replica molding of the master in PDMS and sealing the replica to a glass slide after oxidizing both surfaces with an air plasma.[44] The PDMS separation device was connected to a series of 12 outlet tubings. We used the same tubing for the outlets as for the inlets. All the outlet fluidic connections had the same length in order to minimize the difference in fluidic resistance between the channels. To prevent any leakage, all tubings were sealed using a two-component epoxy (Devcon).

The Collection System. Each polyethylene (PE) outlet tubing was connected to a glass vial (National Scientific Co.) with a septum top via a 16-gauge needle. The PE tubings and needles were sealed with epoxy to the septum to prevent leakage. These vials were placed below the height of the separation channel to permit gravity to assist the collection of the sample.

The Exhaust System. Each vial in the collection system had a second PE tube (PE 90, I.D. 0.86 mm, O.D 1.52 mm) attached by a 20-gauge needle through its septum and also connected to a 10-mL syringe that was open to the atmosphere, i.e., no plunger present. All tubings connecting the collection vials to their exhausts were identical in length. The 10-mL syringes were held at a height similar to that of the injecting 60-mL syringe and above that of the separation device to provide a uniform back-pressure through all the outlet tubings.

Parameter Optimization for the Flowing System by Means of Static-Mode Tests: We used batches of Merrifield resins (75-150 μm diameter, Polymer Laboratories) with different degrees of functionalization (0.38, 1.06, 1.24, or 1.95 mmol Cl/g of polymer). A sample from each batch was placed in an aqueous 250-mM $GdCl_3$ solution in a 4-mL glass vial aligned with the axis of the magnets. In order to make the density of each batch more uniform, the beads were soaked in toluene for 1 hour while being shaken. The spheres were subsequently rinsed in

ethanol and water, dried with nitrogen, and dried in an oven at 120 °C. Some of these spheres then were immersed in an aqueous 250-mM $GdCl_3$ solution in a 4-mL glass vial aligned with the axis of the magnets.

We dyed each batch with a different dye to facilitate visualization of the separation. The beads having 0.38 mmol Cl/g polymer were dyed blue (Oil Blue N; CAS 2646-15-3), those having 1.06 mmol Cl/g polymer were labeled red (Sudan Red 7B; CAS 6368-72-5), those having 1.24 mmol Cl/g polymer were dyed orange (Oil Orange 7078; CAS 842-07-9), and those having 1.95 mmol Cl/g polymer were labeled green (Solvent Green 3; CAS 128-80-3). The spheres were dyed by immersing them in 1 mL of toluene containing ~10 mg of dye, shaking them for 2 hours, then filtering, rinsing with ethanol and water, and drying the beads in an oven at 70°C for 1 hour.

Dynamic, Flowing Separation and Collection: Initially, all the fluidic system, from the inlet tubing to the exhaust, was filled with a 250-mM $GdCl_3$ solution and allowed to establish equilibrium. The dyed Merrifield resins were uniformly suspended by sonication in a solution of 250-mM $GdCl_3$ (25 mL) and TWEEN 20 (50 μL). The spheres were injected using the syringe pump at flow rates between from 0.10–0.25 mL/min. After the entire sample passed through the separation device, we increased the flow rate to 1 mL/min in order to speed the passage of the spheres into the collection vials.

To quantify the number of each type of beads in each vial, we sonicated and shook each vial to evenly disperse the beads within the solution. We removed a 50-μL aliquot from each vial and placed it on a piece of filter paper. After drying the filter paper, we manually counted the number of beads of each type present.