# Supporting Information

## Separation of Nanoparticles in Aqueous Multiphase Systems through Centrifugation

Ozge Akbulut[1], Charles R. Mace[1], Ramses V. Martinez[1], Ashok A. Kumar[2], Zhihong Nie[1], Matthew R. Patton[1], and George M. Whitesides[1,3,4*]

[1]Departments of Chemistry & Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, United States

[2]School of Engineering and Applied Sciences, Harvard University, 29 Oxford Street, Cambridge, MA 02138, United States

[3]Wyss Institute for Biologically Inspired Engineering, Harvard University, 60 Oxford Street, Cambridge, MA 02138, United States

[4]Kavli Institute for Bionano Science and Technology, Harvard University, 29 Oxford Street, Cambridge, MA 02138, United States

[*] Corresponding author email: gwhitesides@gmwgroup.harvard.edu

*Materials and Methods*

**Chemicals.** Brij 35 (MW= ~1,200 g/mol), 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), cetyl trimethylammonium bromide (CTAB), chloroauric acid, silver nitrate, ascorbic acid, sodium borohydride, Ficoll (MW= 400,000) g/mol, poly(2-ethyl-2-oxazoline) (PEOZ) (MW=200,000 g/mol), polyacrylamide (MW=10,000 g/mol), poly(diallyldimethylammonium chloride) (MW=400,000 g/mol), poly(ethylene glycol) (PEG) (MW=20,000 g/mol), polyethyleneimine (PEI) (MW=25,000 g/mol), poly(methacrylic acid sodium salt) (MW=5,000 g/mol), polyvinylpyrrolidone (PVP) (MW=55,000 g/mol) and Pluronic F68 were purchased from Sigma-Aldrich. Poly(acrylic acid) (MW= 450,000 g/mol), poly(allylamine hydrochloride) (MW=60,000 g/mol), and poly(vinyl alcohol) (MW= 3,000 g/mol) were obtained from Polysciences. Dextran (MW= 500,000 g/mol) was purchased from Spectrum Chemical. 1-*O*-octyl-β-D-glucopyranoside was purchased from Calbiochem. Sodium dodecyl sulfate was purchased from J.T. Baker. All chemicals were used without further purification. We used Milli-Q water at pH 5.5 throughout the experiments.

**Preparations of the Aqueous Multiphase Systems (MuPSs).** We prepared stock solutions of polymers and surfactants in water without adding salts or adjusting the pH. We then mixed the appropriate volumes of these solutions by vortexing for thirty seconds and centrifuged the mixtures at 2,000*g* for five minutes to obtain phase separation.

**Measurements of Density.** We determined the density of each stock solution and phase via oscillating U-tube densitometry (Anton Paar DM35N).

**UV-Vis Spectroscopy.** UV-Vis spectroscopy was performed on a Hewlett Packard 8453 Spectrophotometer. After separating nanoparticles in an aqueous MuPS through centrifugation, we collected 60 μL of sample from each layer, and directly analyzed these samples in the

spectrophotometer without dilution. For the measurement of the sediment, almost the entire supernatant was removed by pipetting before resuspending the sediment in a 100 μL of a 10 mg/mL CTAB solution.

**Transmission Electron Microscopy (TEM).** TEM was performed on a JEOL 2100; we used Lacey Formvar/Carbon, 300-mesh, copper TEM grids (Ted Pella, Inc.). For TEM on samples from the top and middle phases, we diluted 50 μL of sample from each phase by adding 150 μL of DI water, centrifuged the solutions at 16,000*g* for ten minutes, and collected 20 μL of concentrated sample from the bottom of tube. We put 2μL of this concentrated solution onto a TEM grid and allowed it to evaporate at ambient conditions. To analyze the particles that sedimented to the bottom of the tube after separation, we decanted almost the entire solution. We suspended the sediment in 50 μL 10 mg/mL CTAB solution, deposited 2 μL of this solution onto a TEM grid, and allowed it to evaporate at ambient conditions.

**Statistics on Populations of Nanoparticles.** We measured the sizes of nanoparticles and counted them by analyzing ten bright field TEM images with even illumination from each solution layer using Image J software (National Institute of Health, USA). The analyzed images were chosen to be as representative of the layer as possible. We collected data from ~1000 particles/layer and calculated their average size; we estimated our error by taking the standard deviation of the sizes measured.

**Rheometry.** The rheometry measurements were carried out on a AR2-G2 stress-controlled rheometer (TA Instruments) at room temperature in the shear-rate range of 1/10 to 1/200 (1/s) at room temperature with a geometry cone/plate 1° 40 mm (part no:S#987620). The viscosity over this range of shear-rates was constant within the sensitivity of the instrument

implying that shear-thinning and shear-thickening effects are minimal for the polymers and surfactants used. The viscosities listed in Table S1 were measured at a shear-rate of 1/100 (1/s).

***Experimental Details***

**Hydrodynamic Behavior of Objects with Different Shapes in a Viscous Medium.** In order to separate objects by shape and size, we exploited the differences in viscous drag that objects experience during centrifugation. The concept is best exemplified by the ratio of the sedimentation coefficients of two different shapes. The sedimentation coefficient $S$ (s) is defined by eqn. 1, with $v_T$ (cm/s) as the terminal velocity of the object in the medium and □ (cm/s) as the acceleration provided by gravity or centrifugation:

$$S = \frac{v_T}{a} \qquad (1)$$

Using Stokes' Law, the sedimentation coefficient for a sphere $S_s$ (s) can be found by eqn. 2, where $\eta$ (P; g/(cm s)) is the viscosity of the medium, $d_H$ (cm) is the diameter of the sphere and $\rho_p$ (g/cm$^3$) and $\rho_l$ (g/cm$^3$) are the densities of the object and the liquid medium, respectively:

$$S_s = \frac{1}{18} {d_H}^2 \frac{(\rho_p - \rho_l)}{\eta} \qquad (2)$$

This equation can be modified to account for arbitrary shapes (eqn. 3), where $f/f_o$ is the frictional coefficient of an arbitrary shape, and usually estimated numerically. The term $d_H$ (cm) is the diameter of a sphere of equivalent volume to the object:

$$S = \frac{1}{18} \left(\frac{f}{f_0}\right)^{-1} {d_H}^2 \frac{(\rho_p - \rho_l)}{\eta} \qquad (3)$$

Hubbard and Douglas calculated the frictional coefficient for a rod with a diameter, $d$ (cm), and a length, $l$ (cm), to be equivalent to eqn. 4 (valid for $0 < l/d < 8$):[1]

$$\frac{f}{f_0} = 0.55\left(\frac{l}{d}\right)^{-1/3}\left(1 + 0.869\left(\frac{l}{d}\right)^{0.76}\right) \qquad (4)$$

**Guidelines to Select a MuPS to Separate Nanoparticles by Rate-Zonal Centrifugation.** Separations by rate-zonal centrifugation rely on the differences in the hydrodynamic behavior of objects under centrifugal force—the sedimentation ratio of two objects must be different than 1. For two objects to sediment through a medium, their densities ($\rho_1$ and $\rho_2$) must each be greater than that of the medium ($\rho_l$). When using a MuPS to perform rate-zonal centrifugation, we must also take into account the interfacial surface energies between phases. The interfacial surface energy of a two phase MuPS, or any aqueous two-phase system, is quite small ($\gamma$, 100 nJ/m$^2$ to mJ/m$^2$).[2] For nanoparticles moving through a MuPS, these seemingly small interfacial energies can pose a non-trivial barrier for continued sedimentation. This energy barrier can be overcome with the appropriate choice of object densities ($\rho_p$), relative centrifugal force (RCF), and particle sizes ($d_H$). We use a simplified comparison of energies to determine the relationship between these variables to ensure that the gravitational energy ($E_g$) is significantly greater than the surface energy ($E_s$).

$$\frac{E_g}{E_s} > 10 \qquad (5)$$

To a first approximation, the energies can be written as follows:

$$E_g \approx (\rho_p - \rho_l)RCF \times g\, {d_H}^4 \qquad (6)$$

$$E_S \approx \gamma {d_H}^2 \qquad (7)$$

For densities in units g/cm$^3$, $g$ in units of m/s$^2$, sizes in units of nm, and interfacial surface energies in units of mJ/m$^2$, we assume that the density of the medium is 1, that the gravitational

constant is 10, and that the interfacial surface energy is 0.0001. Incorporating these assumptions into eqn.'s 6 and 7, we can re-write eqn. 5:

$$\left(\rho_p - 1\right) RCF \; {d_H}^2 \; > 10^{-8} \qquad (8)$$

We can use this equation to estimate the speed of centrifugation required for gold nanoparticles. For example, the RCF must be greater than 8,800*g* for a 25-nm (diameter) gold nanosphere ($d_{gold}$=19.3 g/cm$^3$) in order to overcome the effect of the interfacial surface energy. We chose to use 16,000*g* (achievable in a benchtop centrifuge) to ensure that we were well above the limit and also to reduce the time required to separate the objects. The RCF is inversely related to the time required for an object to sediment a fixed distance for a given viscosity.

**Synthesis of Nanoparticles.** We synthesized gold nanorods according to the seeded growth method developed by Nikoobakht and El-Sayed[3] with minor modifications. We prepared a seed solution by mixing cethyl trimethylammonium bromide (CTAB) solution (2.5 mL, 0.20 M) with 1.5 mL of 1.0 mM $HAuCl_4$. We vigorously stirred this solution and then quickly added 0.60 mL of ice-cold 0.010 M $NaBH_4$ and immediately observed the formation of a brownish-yellow color. The seed solution continued to be stirred for another two minutes and was then kept at 25 °C for thirty minutes for aging. We prepared the growth solution by mixing 50 mL of a 0.2 M CTAB solution with 5 mL of an aqueous 5 mM $HAuCl_4$ solution, 2.8 mL of an aqueous 4 mM $AgNO_3$ solution, and 40 mL of water. Following the addition of 1 mL of an aqueous 0.8 M solution of ascorbic acid, this dark yellow solution turned colorless. Finally, we added 1 mL of an aged seed solution of nanoparticles to the growth solution at 27–30 °C. The color of the solution gradually changed within ten to twenty minutes. The growth medium was kept at constant temperature (27–30 °C) for twenty hours.

After synthesizing gold nanoparticles, we concentrated the products in the reaction solution 20-fold to create a stock solution by centrifuging the total reaction volume for five minutes at 16,000*g* and collecting the concentrated solution of nanoparticles in the bottom by a pipette. This concentrated solution is referred as "suspension of the nanoparticles" in the main text.

**Table S1**. The viscosities of different solutes with densities 1.038 ± 0.001 g/cm$^3$ (± 0.2%).

| solutes | concentration (%w/v) | density (g/cm$^3$) | viscosity (cP) |
|---|---|---|---|
| CHAPS | 24.2 | 1.037 | 2.1 |
| PVP | 20 | 1.038 | 7.4 |
| PEG | 25 | 1.037 | 29.8 |
| Pluronic F68 | 35 | 1.039 | 30.0 |
| PEOZ | 24 | 1.039 | 175.0 |
| PEI | 30 | 1.037 | 302.5 |

**Table S2.** Properties of the aqueous stock solutions of polymers and surfactants that are used in this study: average molecular weight (g/mol), concentration (% w/v or % v/v), density ($g/cm^3$) and viscosity (cP).

| | Polymer | avg. MW (g/mol) | concentration | density ($g/cm^3$)[4] | viscosity (cP) |
|---|---|---|---|---|---|
| 1 | poly(methacrylic acid) | 5,000 | 40 (% w/v) | 1.279 | 684.6 |
| 2 | poly(vinyl alcohol) | 3,000 | 10 (% w/v) | 1.022 | 111.3 |
| 3 | poly(ethylene glycol) | 20,000 | 40 (% w/v) | 1.069 | 284.5 |
| 4 | dextran | 500,000 | 30 (% w/v) | 1.101 | 1071.0 |
| 5 | Ficoll | 400,000 | 40 (% w/v) | 1.130 | 480.0 |
| 6 | poly(diallyldimethyl ammonium chloride) | 400,000 | 20 (% w/v) | 1.044 | 112.6 |
| 7 | polyethyleneimine | 25,000 | 30 (% w/v) | 1.037 | 302.5 |
| 8 | polyallylamine | 60,000 | 20 (% w/v) | 1.052 | 23.7 |
| 9 | Brij 35 | ~1,198 | 30 (% v/v) | 1.025 | 378.0 |
| 10 | 1-*O*-octyl-β-D-glucopyranoside | 292 | 10 (% w/v) | 1.011 | 2.7 |
| 11 | Pluronic F68 | ~8,400 | 44 (% w/v) | 1.049 | 30.0 |
| 12 | CHAPS | 614 | 25 (% w/v) | 1.042 | 4.7 |

**Table S3.** The densities (g/cm$^3$) and viscosities (cP) of dilutions of PEOZ.

| dilutions of PEOZ ( %w/v) | density (g/cm$^3$) | viscosity (cP) |
|---|---|---|
| 35 | 1.059 | 1100.0 |
| 24 | 1.039 | 193.0 |
| 17.5 | 1.028 | 65.1 |
| 12 | 1.017 | 19.1 |
| 8 | 1.012 | 10.8 |
| 4 | 1.004 | 2.5 |

**Figure S1.** Time-dependent penetration of nanoparticles into solutions of PEOZ with different densities and viscosities. In each column, we varied the concentration of PEOZ (35%, 17.5 %, 12%, and 4% (w/v), respectively), while the rows correspond to different time intervals during centrifugation

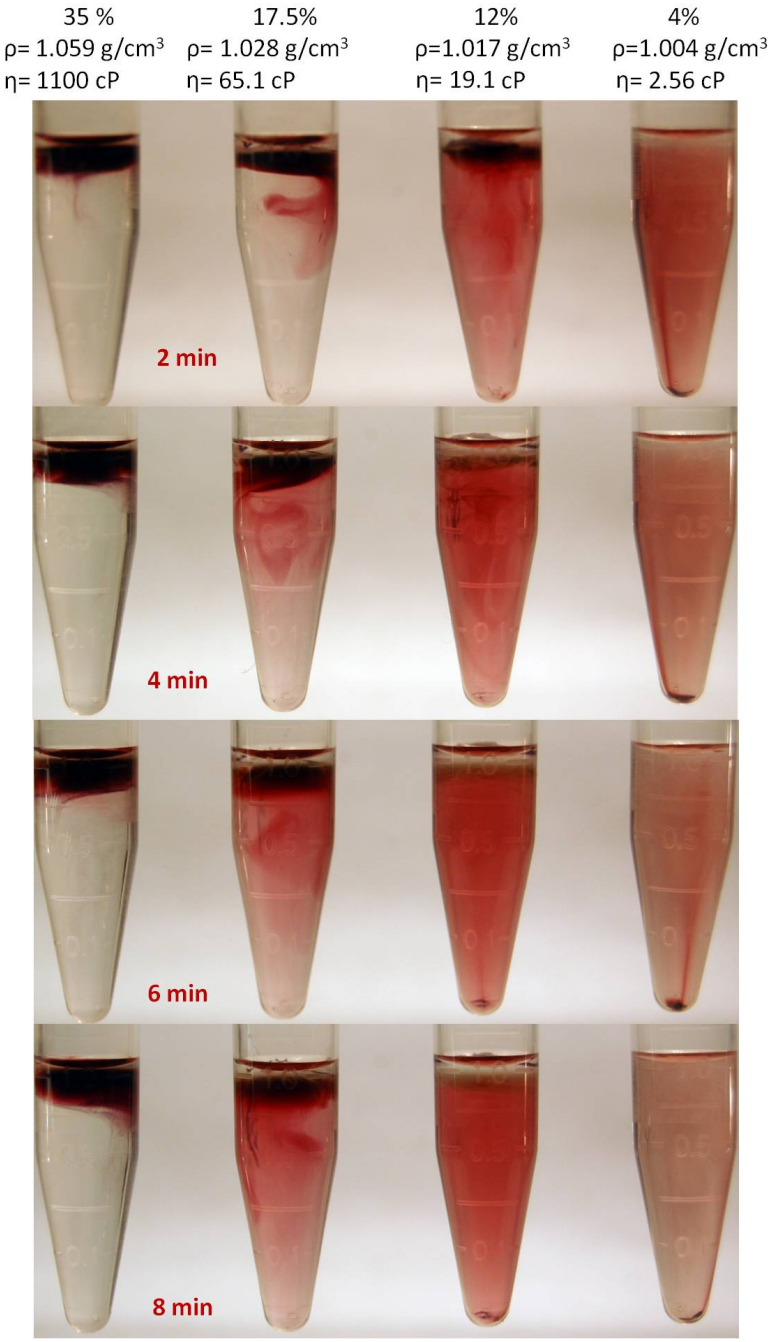
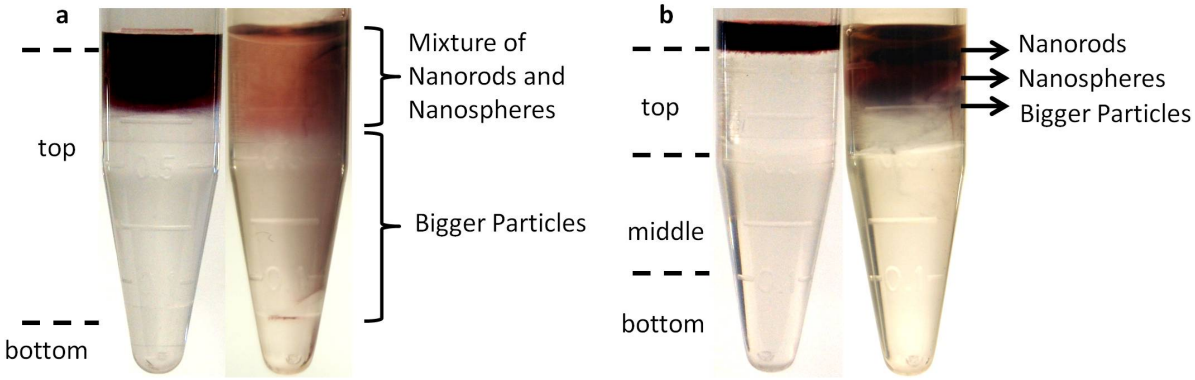


**Figure S2.** Evaluation of aqueous multiphase systems with different viscosities for the top phase. (a) Picture of a two-phase system composed of 1-*O*-octyl-β-D-glucopyranoside (10% w/v) and PEG (40% w/v). The tube on the left shows the system after overlaying the stock solution on top; the tube on the right shows the system after centrifugation at 16,000*g* for two minutes. The viscosity of the top phase of this system is 3.9 cP. (b) Picture of the three-phase system composed of PEG (40% w/v), PEOZ (35% w/v) and dextran (30% w/v). The tube on the left shows the system after overlaying the stock solution on top; the tube on the right shows the system after centrifugation at 16,000*g* for eight minutes. The viscosity of the top phase of this system is 69 cP.



## References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