Separation and purification of nanoparticles in a single step

Supporting Information

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Characterization techniques

UV-vis absorption spectra of Au- and Ag-NPs were obtained using a Thermo Evolution 300 spectrometer with Vision Pro software. The background microemulsion (sample A) spectrum was subtracted from the spectra of samples B and C. For sample D, a water background, and for sample E, an octane background was subtracted. In all cases, samples were diluted by a constant factor of 20 prior to analysis in the background solvent. Stopped-flow measurements (Figure S1) at constant wavelength (512 nm) were carried out on a Hi-Tech Scientific SF-61 single mixing stopped-flow spectrophotometer. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM 1200 EX Mk. 2 microscope operating at 120 kV with attached digital camera. TEM samples were prepared by adding a drop of solution to a carbon-coated TEM grid and removing excess solution to form a thin film. This was then washed in ethanol to remove excess surfactant. $^1$H-NMR spectra were recorded on a JEOL Lambda 300 MHz machine.

Small-angle neutron scattering (SANS) experiments were carried out on the D22 diffractometer at ILL (Grenoble, France) using a neutron wavelength of $\lambda = 10$ Å at two different detector distances to cover a Q range of 0.0024–0.37 Å$^{-1}$. D$_2$O was used to provide contrast against the hydrocarbon components (CTAB, octane, butanol). Absolute intensities (±5%) for I(Q) (cm$^{-1}$) were determined by calibrating the received signal for the incoherent scattering from 1 mm of H$_2$O. For all samples, a suitable solvent background was removed from the data prior to analysis (samples A, B, C, E: octane, sample D: D$_2$O) Data have been fitted using the FISH iterative fitting program$^1$ to models as described below.

The Small Angle X-ray Scattering (SAXS) equipment consisted of a slit-geometry camera with high flux and low background (SAXSess, Anton-Paar, Graz, Austria), connected to an X-ray generator (Philips, PW 1730/10) operating at 40 kV and 50 mA with a sealed-tube Cu anode. A Göbel mirror was used to convert the divergent polychromatic X-ray beam into a focused line-shaped beam of Cu K$_\alpha$ radiation ($\lambda = 0.154$ nm). The 2-dimensional scattering pattern was recorded by a PI-SCX fused fiber optic taper CCD camera (Princeton Instruments - Roper Scientific Inc., Trenton, USA) and integrated into a 1-dimensional scattering function $I(q)$. The temperature of the 1 mm quartz capillary and the
metallic sample holder was controlled by a Peltier element. The samples were thermally equilibrated for 30 min prior to the measurement. Dispersions were exposed to X-rays for 10 min, 3 times for averaging.

Data were processed using the GIFT program to generate pair distance distribution functions (PDDFs) as discussed below.

Figure S1. Stopped flow investigation of the Au-NP formation process, showing plots for relative absorbance at 512 nm against time for the Au synthesis (AuCl$_4^-$ containing ME + NaBH$_4$-containing ME) versus the control (AuCl$_4^-$ containing ME + AuCl$_4^-$ containing ME). Note that the time axis includes a dead-time of 10 ms.
Compositional analysis of separated sample

Upper phase (sample E)

CH₂Cl₂ (2 vol% = 0.6 mol dm⁻³) and a portion of Sample E (upper phase, 25 mg) was diluted to 1 ml in CDCl₃. Peak assignments and integrals are summarized in table S1. By setting the peak integral value from CH₂Cl₂ to 2, each integral value of 1 is standardized to be equivalent to “0.3 mol dm⁻³ H”. The multiplet at 3.65 ppm is indicative of just butanol -O-CH₂- (2H), therefore:

- [butanol] = (0.085/2)*0.3 = 0.013 mol dm⁻³ (0.95 mg ml⁻¹, 4 wt% in upper phase)

Similarly, given the lack of a trimethyl CTAB-headgroup peak in the region 3-3.5 ppm (singlet, integral equivalent to 9H: see Fig S2), the multiplet at 1.26 ppm arises solely from octane -CH₂-, giving:

- [octane] = (8.38 / 12)*0.3 = 0.21 mol dm⁻³ (24 mg ml⁻¹, 96 wt% in upper phase)

Concentrations given are in CDCl₃ solvent (the NMR sample). On the basis that the total concentration of the upper phase in the CDCl₃ was 25 mg ml⁻¹, the lack of evidence for the presence of CTAB and the negligible contribution of water to the peak at 1.55 ppm (on the basis of butanol –CH₂- contribution; see table S1), the sample is almost entirely comprised of octane with a very low concentration of CTAB and water. The butanol : octane volume ratio is now of order 1 : 28, down from 1 : 4 in the initial sample.

<table>
<thead>
<tr>
<th>Peak position / ppm</th>
<th>Multiplicity</th>
<th>Integral</th>
<th>Proton environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>Singlet</td>
<td>-</td>
<td>TMS</td>
</tr>
<tr>
<td>0.88</td>
<td>Triplet</td>
<td>4.327</td>
<td>Octane -CH₃ (6H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Butanol -CH₂ (3H)</td>
</tr>
<tr>
<td>1.26</td>
<td>Multiplet</td>
<td>8.384</td>
<td>Octane -CH₂- (12H)</td>
</tr>
<tr>
<td>1.55</td>
<td>Multiplet</td>
<td>0.170</td>
<td>Butanol -CH₂- (4H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H₂O (2H)</td>
</tr>
<tr>
<td>3.66</td>
<td>Triplet</td>
<td>0.085</td>
<td>Butanol -O-CH₂- (2H)</td>
</tr>
<tr>
<td>5.30</td>
<td>Singlet*</td>
<td>2.000</td>
<td>CH₂Cl₂ (2H)</td>
</tr>
</tbody>
</table>

Table S1. Peaks and integrals table for ¹H-NMR spectrum of sample E, the upper phase. * singlet at 5.30 has satellite peaks at 5.00 and 5.59 ppm
Figure S2. $^1$H-NMR spectrum of sample E, the upper phase, run in CDCl$_3$ doped with 2 vol% CH$_2$Cl$_2$ (peak at 5.30 ppm) and TMS (peak at 0.00 ppm). The expected chemical shift singlet from the 9 protons in the CTAB headgroup is highlighted: this peak is clearly not observed.

**Lower phase (sample D)**

Acetone (1 vol% = 0.136 mol dm$^{-3}$) and a portion of Sample D (lower phase, 25 mg) was diluted to 1 ml in D$_2$O. Peak positions and integral values are summarized in table S2. By setting the integral value of the singlet at 2.19 ppm (acetone, 6H) to 6, each integral value of 1 is standardized to “0.136 mol dm$^{-3}$ H”. The peak at 3.17 ppm (integral value = 0.4445) is solely from the CTAB headgroup protons, therefore

- $[\text{CTAB}] = (0.4445 / 9) \times 0.136 = 0.0067$ mol dm$^{-3}$ (2.5 mg ml$^{-1}$, 10 wt% in lower phase).

To calculate [butanol], it is first necessary to remove the CTAB component of peak at 3.17 ppm = 0.4445 * 2/9 = 0.099, so effective integral is 0.4193. Therefore:
- \([\text{butanol}] = (0.4193 / 2) \times 0.136 = 0.029 \text{ mol dm}^{-3} (2.1 \text{ mg ml}^{-1}, 9 \text{ wt\% in lower phase})

To extract the [octane], it is necessary to remove the contributions to the peak at 1.2 ppm from both butanol and CTAB. The CTAB component of peak around 1.2 is approximately \(0.4445 / 9 \times 26 = 1.284\), the butanol component of peak around 0.85 is approx 0.629, therefore, the corrected integral of the octane peak at 1.2 is 3.9355 (12H), which agrees well with the corrected (by the same process) integral value of the peak at 0.85 ppm of 2.0521 (6H). Therefore:

- \([\text{octane}] = (3.9355 / 12) \times 0.136 = 0.045 \text{ mol dm}^{-3} (5.1 \text{ mg ml}^{-1}, 20 \text{ wt\% in lower phase})

The composition of the lower phase is therefore water-dominated \((25 – (2.5 + 2.1 + 5.1) = 15.3 \text{ mg ml}^{-1} \text{ or } 61 \text{ wt\% of lower phase})\). Using the above concentrations, the molar concentration of CTAB in the lower phase can be estimated as approximately \(0.27 \text{ mol dm}^{-3}\), indicating that almost all of the surfactant not involved in stabilizing Au-NPs has been transferred to the lower phase, and the volume ratio of butanol : octane is now 1 : 2.8 (up from 1 : 4 in the initial sample).
Figure S3. $^1$H-NMR spectrum of the lower phase (sample D) run in D$_2$O doped with 1 vol% acetone-d$_6$ (peak at 2.19 ppm, with satellites at 1.89, 2.39).
### Table S2. Peaks and integrals table for $^1$H-NMR spectrum of Sample D (lower phase).

<table>
<thead>
<tr>
<th>Peak position / ppm</th>
<th>Multiplicity</th>
<th>Integral</th>
<th>Proton environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85</td>
<td>Triplet</td>
<td>4.327</td>
<td>Octane -CH$_3$ (6H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Butanol -CH$_2$ (3H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CTAB -CH$_3$ (3H)</td>
</tr>
<tr>
<td>1.26</td>
<td>Multiplet</td>
<td>8.384</td>
<td>Octane -CH$_2^-$ (12H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CTAB -CH$_2^-$ (24H)</td>
</tr>
<tr>
<td>1.55</td>
<td>Multiplet</td>
<td>0.170</td>
<td>Butanol -CH$_2^-$ (4H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H$_2$O (2H)</td>
</tr>
<tr>
<td>2.19</td>
<td>Singlet</td>
<td>6.000</td>
<td>Acetone (6H)</td>
</tr>
<tr>
<td>3.17</td>
<td>Singlet</td>
<td>0.445</td>
<td>CTAB -N$^+$-(CH$_3$)$_3$ (9H)</td>
</tr>
<tr>
<td>3.56</td>
<td>Triplet</td>
<td>0.085</td>
<td>Butanol -O-CH$_2^-$ (2H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CTAB -CH$_2$-N- (2H)</td>
</tr>
<tr>
<td>4.79</td>
<td>Singlet*</td>
<td>-</td>
<td>D$_2$O residual</td>
</tr>
</tbody>
</table>

*Singlet at 2.19 has satellite peaks at 1.95 and 2.40 ppm*

### SANS model details

All SANS fits have been carried out using the interactive analysis tool FISH$^1$ written by R. K. Heenan employing a standard iterative least-squares method. SANS profiles are fitted to models comprising a form factor, P(Q) which contains information on particle size, shape, polydispersity and concentration, and (where necessary) a structure factor, S(Q) which is related to inter-particle interactions.

The P(Q) for polydisperse spheres each with radius $R$ is defined as follows:$^2$

$$P(Q) = \int_0^{\pi\over 2} |G(Q, R)|^2 g(R) dR$$

(1)

$$G(Q, R) = \left( {4\pi \over 3} \right) R^3 \Delta \rho \left[ {3 j_1(QR) \over QR} \right]$$

(2)
Where $j_l(QR)$ is a first order spherical Bessel function and $g(R)$ is (in this case) defined by a Schultz distribution of homogeneous spheres:

$$g(R) = \frac{\left(\frac{Z+1}{R}\right)^{z+1} R^z \exp\left[-\left(\frac{Z+1}{R}\right) R\right]}{\Gamma(Z+1)}$$

(3)

Where the width parameter, $Z > -1$, $\bar{R}$ is the mean of the distribution and polydispersity defined by an RMS deviation $\sigma = \bar{R} / (Z+1)^{1/2}$. Parameters fit in FISH were sphere radius $R_{av}$, polydispersity index $\sigma/R_{av}$, and the Schultz scale factor given by $SF = 10^{24} \phi (\Delta \rho)^2$

The hard sphere $S(Q)$ model is of the type employed previously by Vrij$^3$ and Kotlarchyk et al.$^4$ Fitted terms are hard sphere volume fraction (set as equal to the aggregate volume fraction) and hard sphere radius.

**Discussion of SANS / SAXS analysis**

For samples A to C, SANS data has been fitted to form factor, $P(Q)$, model representing a Schultz distribution of polydisperse spheres, with hard sphere structure factor, $S(Q)$. The contributions from the $P(Q)$ and $S(Q)$ to the total intensity, $I(Q)$ fit are highlighted on Figure S4. Samples A and B have almost identical $P(Q)$ and $S(Q)$ profiles, indicating little effect of added salt on the microemulsion droplet dimensions. Despite the Au-NPs being larger than the background equilibrium microemulsion droplets, the addition of Au-NPs (sample C) has little effect on $S(Q)$, leading only to a slight increase in $P(Q)$, and therefore a small increase in $I(Q)$. This increased $I(Q)$ almost certainly arises from large Au-NPs, but it is a small overall contribution to the SANS (mostly due to low NP concentration and SANS contrast).

Therefore the use of a fitting model representing a bimodal distribution of small microemulsion droplets and large particles was unable to resolve clearly two distribution envelopes. Fitted parameters to the SANS are summarized in table S3. For all three samples, the Schultz scale, average radius and polydispersity values do not change greatly with composition. A droplet core radius of 15 Å is lower than might be expected for typical $w = [H_2O] / [CTAB] = 10$ microemulsion droplets (e.g. $R = 23$ Å for
water / CTAB / chloroform / isooctane\(^5\), \(R = 22 \, \text{Å}\) for water / AOT / n-heptane\(^6\)). In addition, the fitted scale factor (SF) is low in comparison to expected value (approximately \(3.33 \times 10^{-4}\) calculated on the basis of a sharp contrast step defined as \(\Delta \rho = \rho_{\text{octane}} - \rho_{\text{D}_2\text{O}}\) and the volume fraction of added \(\text{D}_2\text{O}, \phi_{\text{D}_2\text{O}} = 0.072\)). These discrepancies could be explained by the presence of the alcohol co-surfactant; for AOT-stabilized systems, addition of co-surfactant has been shown to favor the formation of larger numbers of smaller droplets.\(^7,8\) In addition, H-D exchange\(^7\) from alcohol co-surfactant (H) to \(\text{D}_2\text{O}\) in droplet cores has previously been suggested to account for reduced SANS intensity, borne out by a lower scale factor, due to a reduction in contrast. This latter argument is particularly compelling given the high butanol level (\(\phi = 0.2\)) compared to \(\text{D}_2\text{O}\) (\(\phi = 0.072\)). The radius and volume fractions suggested by the hard-sphere \(S(Q)\) model are likely to reflect the entire droplet (e.g. including any interfacial species – CTAB or butanol) and hence are larger than the values from the \(P(Q)\) fit (for droplet \(\text{D}_2\text{O}\) core only). The difference between the \(R_{S(Q)}\) and \(R_{\text{av}}\) therefore gives the surfactant / co-surfactant shell thickness, for which a calculated value of 13 - 14 Å is sensible and agrees with previously reported values.\(^9\) It should be noted that \(\phi_{S(Q)}\) for all samples A-C is lower than the theoretical maximum calculated by summation of \(\phi_{\text{D}_2\text{O}} + \phi_{\text{CTAB}}\). This suggests that not all of the CTAB is present at the interface.

In all cases, SAXS data have been fitted by a generalized indirect Fourier transform (GIFT) method,\(^10,11\) using a model-free determination of \(P(Q)\), and a hard sphere interaction model for \(S(Q)\). This analysis method generates a pair distance distribution function (PDDF) shown for samples A-C in figure S5. The intersection with the x-axis gives the maximum diameter \(D_{\text{max}}\),\(^10-12\) and if the scattering species are assumed to be spherical (a reasonable assumption of the basis of the shape of both SANS and SAXS profiles) the position of the peak in the PDDF is indicative of a most probable particle or droplet radius.\(^13\) It is clear from figure S6 that the microemulsion droplets in samples A and B have very similar dimensions, in agreement with SANS analysis. However, the average droplet radius (21 Å for both samples) is higher than that calculated by SANS, and more in agreement with previous CTAB-stabilized microemulsion droplet radii found before (as listed above). This discrepancy probably reflects more the advantages of using SAXS in this case; any problems with H-D exchange are negated as there
is no difference in SAXS contrast between H and D. In addition, as discussed in the main paper, accurate model fitting is problematic when systems have high polydispersity, concentration or large interparticle interactions. Such problems are not experienced to such a high degree using model-free analysis methods, such as GIFT.

As reflected in the extracted functions, the difference between the PDDF of sample C and samples A, B is clearly evident. The function appears to have two maxima; as might be expected given the presence of Au-NPs. Despite the low concentration of Au-NPs, the particles were seen by TEM (Figure 2 in the main paper), to be much larger than the background microemulsion droplets (as determined by SAXS, SANS). In addition, the scattering cross section of gold for x-rays is large, so the SAXS contrast against the mainly hydrocarbon solvent is high. In order to test for a bimodal distribution of microemulsion droplets and Au-NPs, an intensity-weighted size distribution was calculated using GIFT. Scattered intensity scales with the $6^{th}$ power of the radius, hence using this weighting the small concentration of larger Au-NPs becomes apparent and the mean values are higher. For comparison, this analysis was also carried out on the data from sample B and the two profiles are shown on Figure S6. The distribution for sample C is clearly bimodal; with one species (presumably microemulsion droplets) of order the same dimensions ($\sim 24 \text{ Å}$) and distribution as sample B and one (presumably Au-NPs) much larger, with an average radius of order 52 Å.

The SANS data from sample E were fitted to a simple form factor model describing a Schultz distribution of polydisperse spheres, as used for the microemulsion samples. Fitted parameters were $\text{scale} = 5.4 \times 10^{-7}$, $R_{av} = 20 \pm 4 \text{ Å}$, $\sigma/R_{av} = 0.2$. The only conclusion that can reliably be made is that the SANS is indicative of a small number of small water droplets. The extremely low value for the scale factor indicates the concentration of droplets is very low (if $\Delta \rho = \rho_{\text{octane}} - \rho_{\text{D}_2\text{O}}$, $\phi = 0.0001$, although as already discussed H-D exchange from D$_2$O with the labile proton in butanol is likely to lead to a smaller value for the true contrast step $\Delta \rho$, and hence a large volume fraction $\phi$).

SAXS results were analyzed using GIFT and the pair distance distribution function was again calculated and is shown in figure S7. The average radius (if the Au-NPs are be modeled as polydisperse
spheres) is 45 Å, with a maximum dimension of 130 Å. This value of $$R_{av}$$ is in good agreement with the value obtained for the larger dimensions (assumed to be Au-NPs) found using the intensity distribution analysis of sample C described above ($$R_{av} = 52$$ Å), but is smaller than the size ranges noted by TEM (100 – 400 Å, see main paper). Whilst it is likely that Au NPs are not all spherical (figure 2 main paper), and so the assumption that the sample consists of spherical particles represents an approximation, it is possible that additional aggregation has taken place on the TEM grid during the preparation process further enhancing the differences.

The S(Q) functions extracted from the SAXS data for all samples A – E is shown in figure S8; the S(Q) for sample D is much more prominent than for any of the other samples. Fit parameters of $$\phi = 0.407$$, $$R = 81$$ Å, mean deviation = 30 Å echo the results in the PDDF and point to the former explanation of sample composition (oil-swollen micelles).

![Figure S4. The separate form factor P(Q) and structure factor S(Q) components to the model fits for SANS data shown in figure 4 (main paper) for samples A-C. P(Q) represents a Schultz distribution of polydisperse spheres, S(Q) is an effective hard sphere structure factor.](image-url)
<table>
<thead>
<tr>
<th>Sample</th>
<th>Form Factor, P(Q)</th>
<th>Structure Factor, S(Q)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scale</td>
<td>Radius</td>
</tr>
<tr>
<td>(A) Blank ME</td>
<td>$1.37 \times 10^{-4}$</td>
<td>15</td>
</tr>
<tr>
<td>(B) ME + AuCl₄</td>
<td>$1.37 \times 10^{-4}$</td>
<td>15</td>
</tr>
<tr>
<td>(C) ME + Au-NPs</td>
<td>$1.39 \times 10^{-4}$</td>
<td>15</td>
</tr>
</tbody>
</table>

Table S3. Parameters from model fits to SANS data for samples A-C as shown in figure 4 (main paper).

Note that $\phi_{D2O} \approx 0.07$, $\phi_{CTAB} \approx 0.14$ in samples A-C, so theoretical $\phi_{S(Q)} \approx 0.21$.

Figure S5. PDDFs for samples A – C, normalized to unity. The intersection with the x-axis gives the maximum particle / droplet diameter.
Figure S6. Intensity distribution function for samples B and C normalized to unity, showing the bimodal distribution of microemulsion droplets and Au-NPs in sample C.

Figure S7. PDDFs for samples D and E, normalized to unity.
Figure S8 Plot of $S(Q)$ functions from fits to SAXS data for samples A – E, showing a large $S(Q)$ for sample D in comparison with other samples.
References


(12) Glatter, O. J. Appl. Cryst. 1979, 12, 166-175.