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One-Pot Synthesis with *in situ* Preconcentration of Spherical Monodispersed Gold Nanoparticles using Thermoresponsive 3-(Alkyldimethylammonio)-propyl Sulfate Zwitterionic Surfactants

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Experimental Section

Instrumentation

Ultraviolet-visible spectra were obtained using a JASCO V-650 double beam spectrophotometer equipped with a 1.7-mL quartz cuvette (1-cm light path length × 0.5-cm internal cuvette width). For some measurements, a special SAH-769 accessory (1.0 mm light path disk cell) was attached to the spectrophotometer in order to measure micro-volume samples (minimum volume of 5.0 μL). This attachment was employed to measure the spectra of some small volume surfactant-rich phase samples directly without the need for dilution.

Dynamic light-scattering (DLS) measurements were performed using a HORIBA SZ-100 nanoparticle analyzer using a fluorescence analytical grade quartz cuvette (1-cm × 1-cm) at room temperature (25.0 ºC) with the green laser output at 532 nm and the measurement scattering angle set to 90.0º. DLS measurement of particle size is strongly influenced by the viscosity of sample solution. Thus, a sine-wave vibro viscometer SV-A (A&D Co. Ltd., Tokyo, Japan) was used to measure the viscosity of the sample solutions prior to the DLS measurement. The viscosity was then imputed as the parameter of DLS measurement. The hydrodynamic diameters reported are the mean diameters.

Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-1010 transmission electron microscope (TEM; Tokyo, Japan) operating at 80kV. TEM samples were prepared by placing 5.0 μL aliquots of the aqueous sample or the diluted surfactant-rich phase (which had been diluted by a factor of 11 with methanol) onto a carbon-filmed 200-mesh Cu grid (Nisshin EM Co. Ltd., Tokyo, Japan). After sample loading, the grids were dried at room temperature prior to the TEM measurements. The AuNP size distribution was typically determined by measuring 50 particles.

The zeta potential measurements were made employing a Nano Z ZEN3600 Zetasizer (Malvern Instruments Ltd, Worcestershire, UK).

A Bruker micro time-of-flight mass spectrometer (TOF-MS) was employed to measure the mass spectrum of the zwitterionic surfactants. Infrared spectra (IR) were obtained using a Spectrum Two instrument with an ATR attachment.

The pH measurements were made using a HORIBA handy D-50 pH meter equipped with a long ToupH combination 9680-10D electrode. This electrode probe was employed to directly measure solution pH in the conical-shaped centrifuge tube.

A Kubota 2410 centrifuge (Tokyo, Japan) was employed in the centrifugation step in order to facilitate phase separation and isolation of the surfactant-rich phase containing the AuNPs.

Materials

Hydrogen tetrachloroaurate trihydrate, $HAuCl_4 \cdot 3H_2O$ and trimethylene sulfate were obtained from Sigma-Aldrich (St. Louis, MO, USA) while sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), *L*(+)-sodium ascorbate ($NaC_6H_7O_6$), sodium chloride (NaCl), sodium hydroxide and hydrochloric acid were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Analytical grade diethyl ether and HPLC grade methanol were both obtained from Nacalai Tesque Inc. Ltd. (Kyoto, Japan). All of these materials were used as received without any further purification. Ultrapure water was prepared using a Direct Milli-Q 3UV Water System (Millipore, Corp., Milford, MA).

The zwitterionic surfactants, C_9APSO_4 and $C_{10}APSO_4$ (catalog # 472603 and 47258-1, respectively) were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification [note: these surfactants are no longer available from Sigma-Aldrich]. These surfactants can also be prepared using published literature methods.[1,2] Briefly, the appropriate alkyldimethylamine was mixed with trimethylene sulfate in dry acetone and gently heated. The surfactant products were recrystallization, washed with dry ether and dried in a vacuum desiccator at room temperature. The required alkyldimethylamines, dimethylnonylamine and dimethyldecylamine, were synthesized according to the procedure reported by Saitoh and Hinze.[2] The mass, infrared and NMR spectrum of the synthesized C_{10}-$APSO_4$ surfactant are shown in Figures S1, S2 and S3, respectively. The spectral features of the synthesized surfactants were in good agreement with that of the commercial product.

Solutions and Procedures

Glassware Cleaning Protocol.
All glassware utilized in the synthesis of the AuNP were successively washed with detergent, saturated I_2 - KI solution, 1 M nitric acid solution, methanol and finally acetone. The glassware were then dried in an oven at 105 ^oC overnight (ca. 12 hours).

Preparation of Concentrated Stock Solutions of the Zwitterionic Surfactants.
Appropriate amounts of the C_9APSO_4 and $C_{10}APSO_4$ surfactants were transferred to volumetric flasks and diluted to the mark with ultrapure water at room temperature. Since room temperature is below the upper critical solution temperature (UCST), this solution will be heterogeneous and will appear turbid or cloudy. However, when heated about their UCST, clear homogeneous solutions will result which were then utilized in the AuNP synthesis. Note that long term storage of these zwitterionic surfactant stock solutions under acidic conditions, particularly at elevated temperatures, should be avoid since they will hydrolyze[3] in a similar fashion to anionic alkyl sulfate surfactants (as sodium dodecylsulfate).[4]

General Experimental Procedure for the Synthesis of AuNP.
The typical synthetic protocol consisted of transferring 0.50 mL of an aqueous 0.010 M trisodium citrate solution and 1.00 mL of an aqueous 10 % (w/w) zwitterionic $C_{10}APSO_4$ ($C_{10}H_{21}(CH_3)_2N^+(CH_2)_3OSO_3^-$) solution (both preheated to 95.0 ^oC in a thermostatted oil bath) to

a 5.00 mL conical bottom centrifuge tube (Pyrex brand, with graduations) and mixing to prepare a homogeneous solution. This solution was adjusted to pH 7.0 via addition of appropriate amounts of 0.10 M NaOH (typically 0.10 mL was required). This tube and its contents were kept in the 95.0 ^{o}C oil bath. Lastly, the synthetic reaction was initiated by addition of 2.50 mL of a 1.02×10^{-4} M $HAuCl_4$ solution (at room temperatue) to the aforementioned tube and after mixing, this reaction mixture was kept static in the 95.0 ^{o}C thermostatted oil bath for 8.0 minutes. The tube was then removed from the oil bath and after 2-3 minutes, placed in a centrifuge and centrifuged for 8 min. at 1500 rpm (x 367.1 g) at room temperature. [Note: excess centrifugation should be avoided as the AuNPs can agglomerate, aggregate and/or form other states]. During this step, the solution cooled to room temperature (which is below the surfactant's UCST) and phase separated. The AuNPs were found to be concentrated in the bottom surfactant-rich phase whose volume was 90 μL. The top surfactant-lean phase was completely removed using a Pasteur pipette. The bottom surfactant-rich phase containing the AuNPs was kept in the tube for storage or appropriately diluted with methanol prior to characterization studies.

The same general experimental procedure just outlined was employed in the studies designed to evaluate the influence of the different reaction variables upon the formed AuNPs. For instance, in the study of effect of $C_{10}APSO_4$ concentration, all steps and conditions were the same except that the concentration of the $C_{10}APSO_4$ stock solution was appropriately adjusted so that the desired final surfactant concentration was achieved for the synthesis. Likewise, for the experiments designed to determine the impact of temperature, all experimental steps were the same except for the temperature to which the surfactant and citrate solutions were preheated and at which the reaction was conducted. For the experiments designed to determine the effect of different gold precursor concentrations, all steps in the general protocol were the same except that the $[citrate]_f$ = 2.5 mM, $[C_{10}APSO_4]$ = 5.0% and the concentration of the $AuCl_4^-$ stock solution added to initiate the synthetic reaction was varied.

Determination of the Concentration of the Synthesized AuNP.

The concentration of Au-NPs in solution was calculated based on the Leff equation:[5]

$$n = \left(\frac{4\pi(R-\delta)^3}{3V_g}\right)\Big/V \qquad \text{(eq. 1)}$$

where n is the number of Au atoms in the nanoparticle, R is the radius of AuNP, δ is the length of the capping agent/ ligand (in this case, R >> δ), Vg is the volume of one Au atom (17 Å) and V (mL) is the volume of solvent.

Determination of Zeta Potential.

The zeta-potential of the AuNPs in surfactant-rich phase or the surfactant-rich phase alone were determined by an extrapolation method. Namely, the surfactant-rich phases were diluted with a water-methanol mixed solvent system containing different amounts of methanol and for each, the zeta potential was measured. A plot of the apparent zeta potential versus the methanol

content was constructed and found to be linear. The zeta potential of the surfactant-rich phase or AuNP in the surfactant-rich phase was taken to be its extrapolated value for 0% methanol.

Determination of the Volume of the Surfactant-Rich Phase as a Function of the Total Surfactant Concentration.

The general synthetic procedure as noted in the text was employed except that different concentrations of the C_9APSO_4 and $C_{10}APSO_4$ zwitterionic surfactants were employed and at the conclusion of the centrifugation step, the volume of the surfactant-rich phase (bottom phase) in the conical centrifuge tube was measured at room temperature. The dependence of the volume of the surfactant-rich phase upon the final concentration of surfactant present is shown in Figure S4 under the conditions of the synthetic procedure (centrifugation for 8.0 min. at 1500 rpm at room temperature). It is important to note that this surfactant-rich phase volume is dependent upon the centrifugation time, the centrifugal force generated and temperature at which the centrifugation step is conducted. In addition, it depends upon the presence of other species, such as citrate ions.

Supporting Figures and Discussion

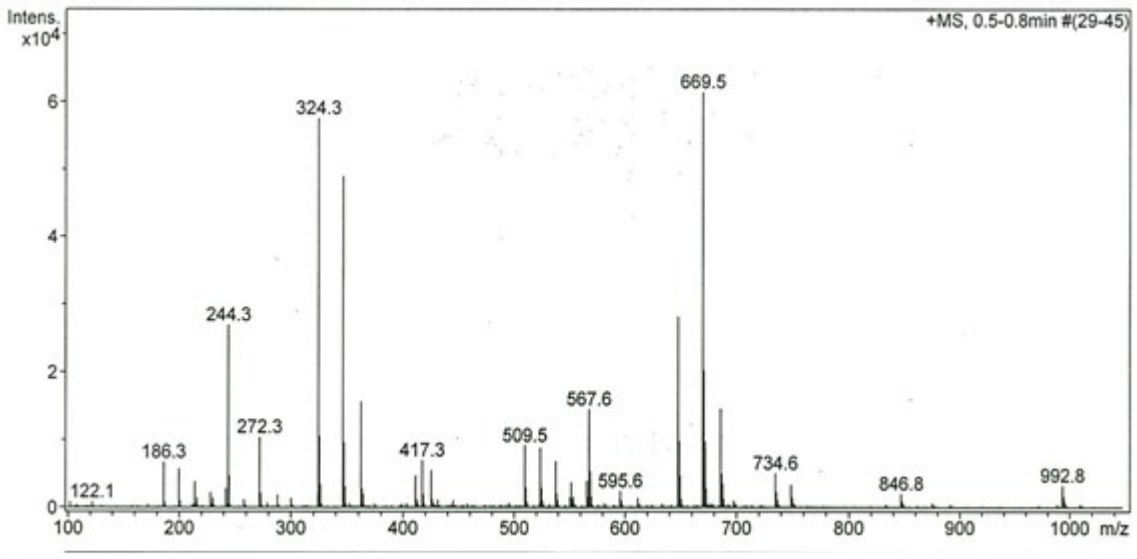

Fig. S1. Mass spectrum of $C_{10}APSO_4$.

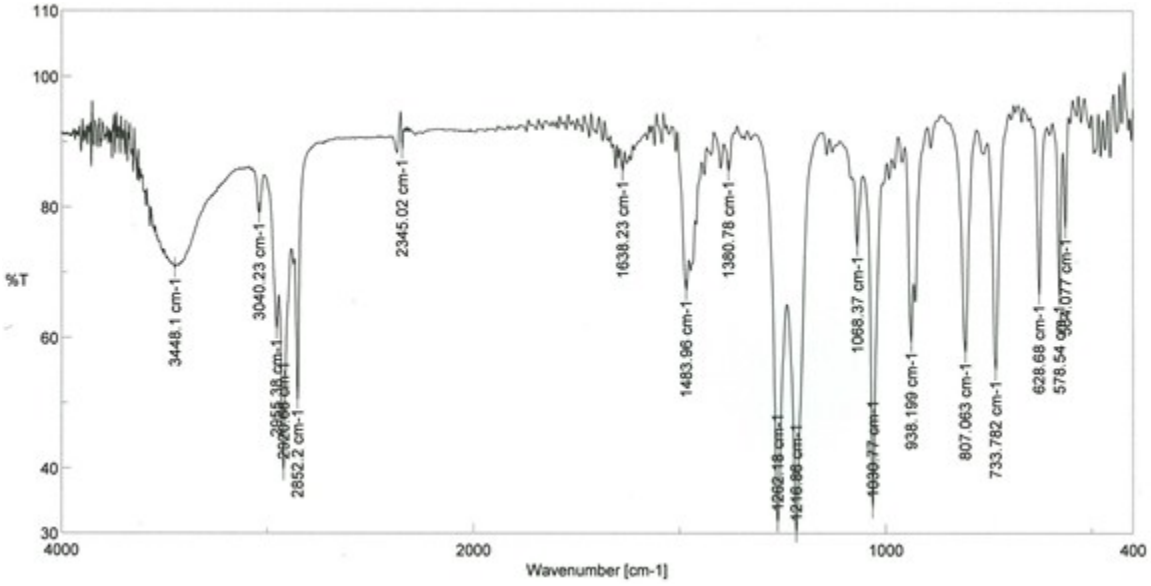

Fig. S2. Infrared spectrum of $C_{10}APSO_4$.

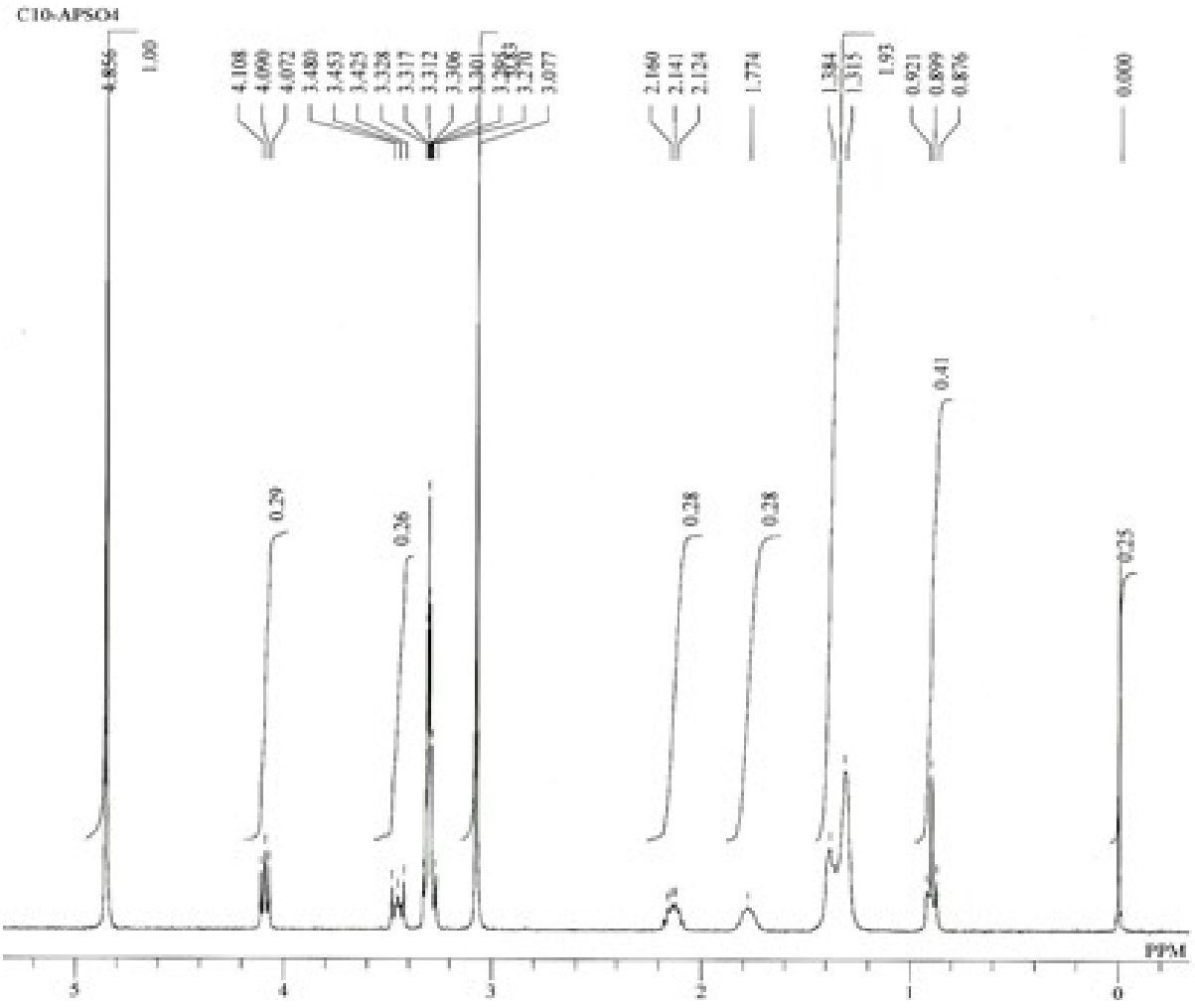

Fig. S3. H-NMR spectrum of $C_{10}APSO_4$.

Partitioning of AuNPs into Surfactant-Rich Phase.

These zwitterionic surfactants and their phase separation behavior have previously been employed for the extraction of hydrophobic organic compounds, the separation of hydrophobic from hydrophilic proteins and the extractive enrichment (preconcentration) of hydrophobic proteins and biological species.[2,6] In the context of that work, it was shown that the bottom, small volume, more dense and viscous phase in the phase separated mixture was the surfactant-rich phase. Any species that binds (or partitions) to the zwitterionic surfactant micelle in the initial homogeneous solution was subsequently found to be concentrated in that surfactant-rich phase following phase separation.[2,6] With regard to the present work, it is thought that the $C_{10}APSO_4$ or C_9APSO_4 can directly bind the AuNPs as they form during the synthesis and/or to the citrate ion present on any citrated capped AuNPs. It has been previously shown for many other zwitterionic micelles that their headgroup can bind anions.[7] Thus, since the AuNPs are bound to the zwitterionic surfactant present in the system, they end up being concentrated in the subsequent surfactant-rich aggregate (coacervate) phase following phase separation. It should be noted that this surfactant-rich phase has a maximum capacity for AuNPs and if that capacity is exceeded, the excess AuNPs do not partition to that surfactant-rich phase but instead remain in the larger volume surfactant-lean phase (see Fig. S4). In this latter case, the AuNPs are excluded from the surfactant-rich phase most likely due to so called excluded volume effect.[8]

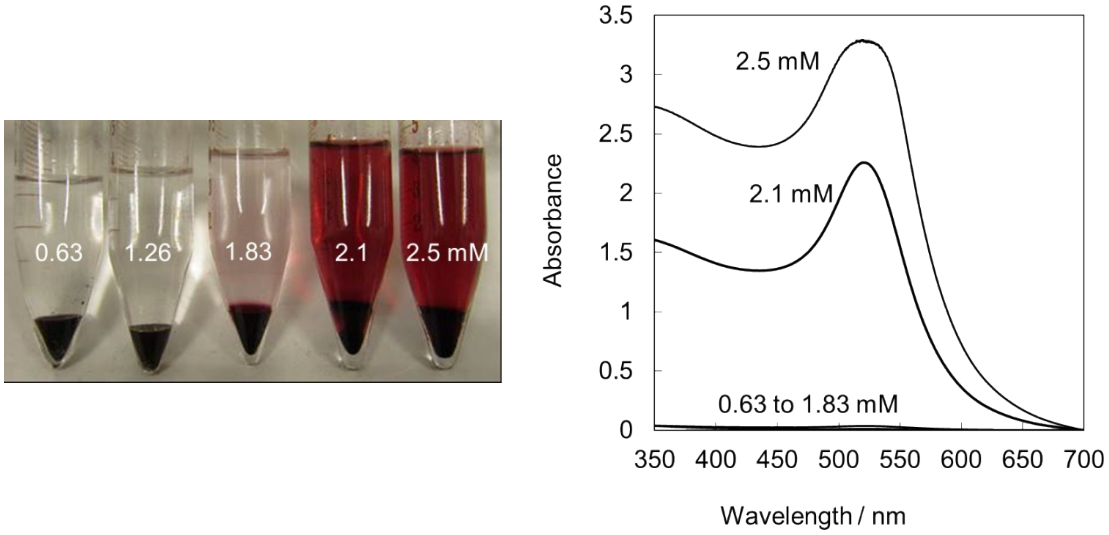

Fig. S4. Left-panel shows the centrifuge tubes for the capacity experiments in which the general protocol was employed except that the concentration of the $AuCl_4^-$ stock solution was progressively increased from 0.63 to 2.5 mM. In the first three tubes, the synthesized AuNPs were all concentrated in the small volume surfactant-rich phase at the bottom of the centrifuge tube. From this experiment, the maximum capacity of AuNPs in the surfactant-rich phase was determined to be 1.77×10^{14} particles/mL (equivalent to 2.91×10^{-7} M AuNP). This capacity can be exceeded without any aggregation in the system as the additional AuNPs formed at greater gold precursor concentrations remain in the upper, larger volume surfactant-lean phase (rightmost two tubes). This might be due to an excluded volume effect.[8] The right-hand panel

shows the absorbance spectra with the AuNP SPR band for the *undiluted upper surfactant-lean phase* volume of each tube's solution. This indicates that the AuNPs formed in excess of the capacity of the surfactant-rich phase remain the upper, surfactant-lean phase and no aggregation is observed in either the surfactant-rich or surfactant-lean phase. Such behavior is in contrast to the situation which occurs in many other AuNP synthetic or preconcentration systems in which agglomeration or aggregation of the AuNP is observed when the maximum capacity of the solvent or phase is exceeded.

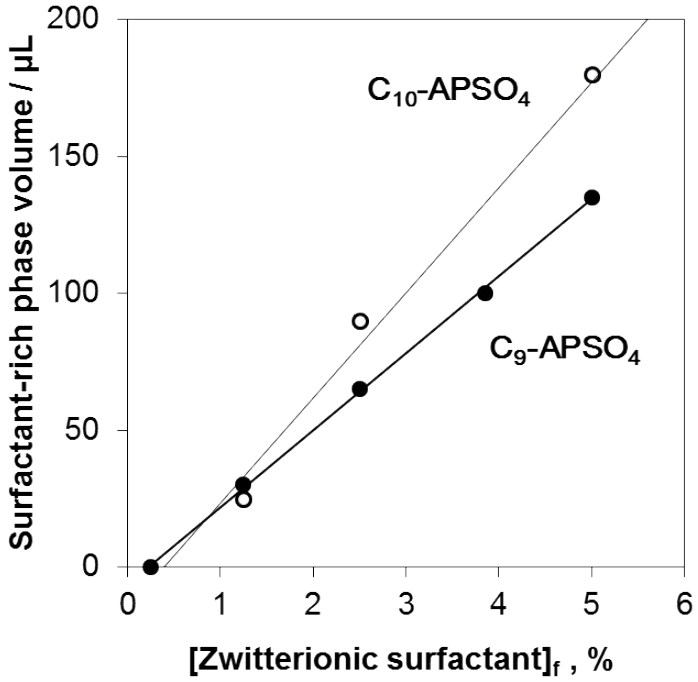

Fig. S5. Dependence of the volume of the surfactant-rich phases of C_9APSO_4 (solid circles) and $C_{10}APSO_4$ (open circles) as a function of the total initial zwitterionic surfactant concentration. The conical centrifuge tubes were centrifuged for 8.0 min. at 1500 rpm (centrifugal force = x 367.1g) at room temperature. [Note: The volume of the surfactant-rich phase depends upon the time of centrifugation as well as the temperature at which it is conducted and centrifugal force generated. In addition, the presence and concentration of ions, such as citrate, influence the volume of the surfactant-rich phase.]

Effect of $C_{10}APSO_4$ Surfactant Concentration upon AuNP Size.

At first, it was surprising that the size of the AuNPs synthesized in 2.50, 5.00 and 7.50% $C_{10}APSO_4$ were essentially the same in view of the literature which indicated that the size of AuNPs (as well as other metal nanoparticles) is impacted by the surfactant concentraton.[9] Depending upon the specific nanoparticle preparation method and type of surfactant organized system employed in the nanoparticle synthesis, the general trends are either an increase in the nanoparticle size with surfactant concentration or a decrease in size with surfactant concentration. The latter is the more frequently reported trend. Careful examination of the reported work indicated that in some instances, the nanoparticle size did not appreciable change with surfactant concentration over a wide concentration interval at the higher surfactant concentrations and only increased in size at lower surfactant concentrations.[9e,9g] Thus, our results seem to mimic such behavior in that the same approximate AuNP size was observed at the higher $C_{10}APSO_4$ concentrations ($\geq$ 2.50%) examined while flocculation/aggregation was observed at the lowest concentration (1.25%).

SPR Absorption Bands for AuNPs Synthesized in $C_{10}APSO_4$ and C_9APSO_4.

At first it seemed surprising that the 21.1 nm sized AuNPs formed in $C_{10}APSO_4$ and the 13.4 nm sized AuNPs formed in the presence of C_9APSO_4 both exhibited their SPR maximum absorption at 529.5 nm (Fig. 3, left panel). However, literature suggests that this is not out of line with what has been previously reported.[10] For instance, the plasmon resonance peak for AuNPs (prepared by citrate method) in water for 12 to 25 nm sized AuNPs all appear to be around ca. 525 nm.[10a] In addition, El-Sayed has reported that for citrate coated AuNPs in aqueous solution the SPR absorption band for 14.8 nm sized particles was 520 nm while that for 21.7 nm sized particles was 521 nm.[10b] Likewise, a manufacturer, Nanocomposix, states that for citrate capped AuNPs in water, the maximum SPR bands appear at 519, 519 and 520 nm for 13.4 nm, 19.7 nm and 29.6 nm sized AuNPs, respectively.[10c] The position and intensity of the SPR band of nanoparticles is known to be dependent upon the dielectric of the medium it is in as well as the refractive index of species (protective capping or other additives) near the particle surface.[11] For AuNPs stabilized with the surfactant, hexadecyltrimethylammonium bromide, CTAB, 13.3 nm sized particles absorbed at 531 nm while 32.2 nm sized particles absorbed at 534 nm.[10d] Thus, the fact that the different sized AuNPs prepared using these two different zwitterionic surfactants appears reasonable given this prior literature precedent and that the resolution of our spectrophotometer was 2 nm.

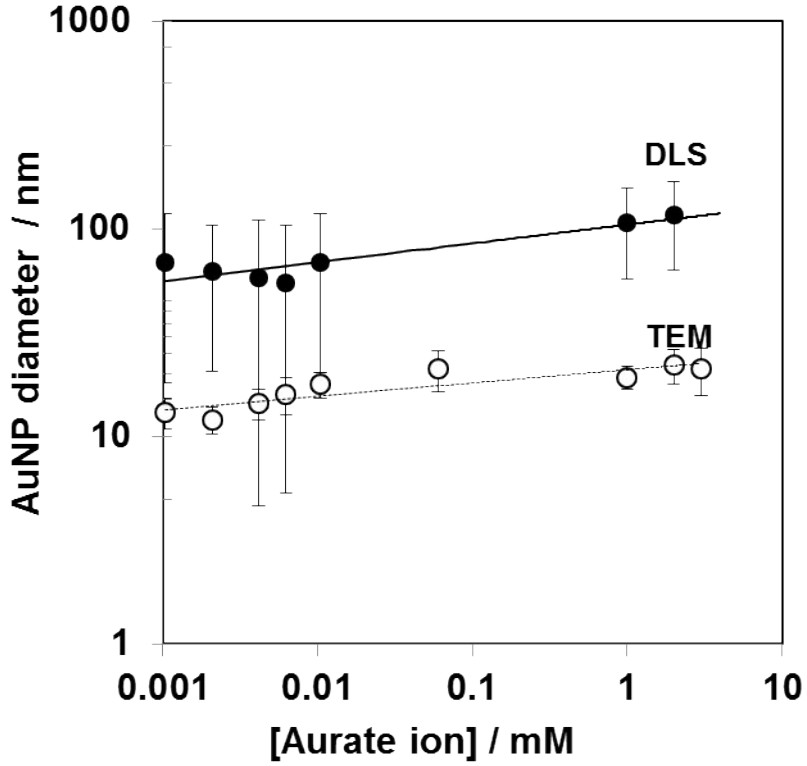

Fig. S6. Plots of the dependence of the AuNP size (diameter as determined from TEM images (open circle) or hydrodynamic diameter from DLS profiles (solid circle)) upon the concentration $AuCl_4^-$ at a fixed citrate concentration of 2.5 mM and $[C_{10}APSO_4]_f$ = 5.0% for the synthetic protocol.

The diameters measured by TEM were consistently smaller than the hydrodynamic diameters measured by DLS (in solution). However, the general trends in sizes and size distributions found from the TEM data correlated very well with those observed by DLS. The literature reports similar instances in regards to AuNP size measurements using TME images versus DLS profiles.[12]

Suitability and Potential Applications of the Synthesized AuNPs.

The synthesized condensed AuNPs are expected to be potentially useful for catalysis applications among others either by use "as is" or after appropriate dispersion in other solvent systems. For instance, it has been reported that 15 nm sized AuNPs coated with the surfactant hexadecyltrimethylammonium bromide (CTAB) or capped with chitosan exhibited essentially the same effect for the catalytic reduction of p-nitrophenol as that of citrate coated AuNPs.[13a,13b] In addition, palladium nanoparticles coated with the zwitterionic surfactant 3-(1-dodecyl-3-imidazolio)propanesulfonate were utilized as a catalyst for the hydrogenation of cyclohexene.[13c] Thus, it appears that these $C_{10}APSO_4$ or C_9APSO_4 coated AuNPs should have similar catalytic ability without any need to remove the zwitterionic surfactant coating.

In addition, the surfactant-rich phase containing the AuNPs could be diluted with water ***if*** an appropriate additive, such as octyl-β-D-glucoside, were added to depress the $C_{10}APSO_4$ UCST to or below room temperature such that one would then have a homogeneous solution[2,6] for the desired application. Alternatively, the surfactant-rich phase could be diluted with other solvents or mixtures of solvents. Experiments indicated that the AuNPs in the $C_{10}APSO_4$ surfactant-rich phase could be successfully dispersed in methanol (MeOH) or in 66.7:33.3 (v/v) MeOH:water and in 40:60 (v/v) MeOH:water mixtures. It is anticipated that they could also be dispersed in dimethyl sulfoxide (DMSO) (an organic solvent sanctioned by the United States Food & Drug Administration for carrier drug studies) as a literature report has noted that AuNPs coated with triphenylphosphoniopropylthiosulfate zwitterions could be dispersed in DMSO.[14]

If a desired application required the removal of the zwitterionic coating from the AuNP, then this could probably be achieved via use of a column chromatographic separation after dilution of the surfactant-rich phase with methanol. It might also be possible to remove the zwitterionic detergent from the AuNPs by dialysis since it had been reported that dialysis is more effective in surfactant removal from proteins when the critical micelle concentration of the surfactant present is high[15] (i.e., greater than 1 mM) as is the case for C_9APSO_4 and $C_{10}APSO_4$.

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