**Prospecting nanomaterials in aqueous environments by cloud-point extraction coupled
with transmission electron microscopy**

Yu Yang^a, Robert Reed^a, Jared Schoepf^b, Kiril Hristovski^c, Pierre Herckes^d, Paul Westerhoff^{a*}

Affiliations:

^aSchool of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, Arizona, 85287-3005, United States

^bSchool for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, Arizona, 85287-6106, United States

^cThe Polytechnic School, Arizona State University, Mesa, Arizona, 85212, United States

^dSchool of Molecular Sciences, Arizona State University, Tempe, Arizona, 85287-1604, United States

***Corresponding Author and Address:**

Paul Westerhoff, School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, Arizona 85287-3005, United States

Phone: 480 965-2885

E-mail: p.westerhoff@asu.edu

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Abstract

Increasing application of engineered nanomaterials (ENMs) in industry and consumer products inevitably lead to their release into and impact on aquatic environments. To characterize the NMs efficiently in surface water, a fast and simple method is needed to separate and concentrate nanomaterials from the aqueous matrix without altering their shape and size. Applying cloud-point extraction (CPE) using the surfactant Triton 114 to an array of NMs (titanium dioxide, gold, silver, and silicon dioxide) with different sizes or capping agents in nanopure water resulted in extraction efficiency of 83%-107%. Additional CPE experiments were conducted to extract NMs from surface, potable, and sewage waters, and NMs enriched in the surfactant phase were characterized using transmission electron microscopy coupled with energy dispersive x-ray spectroscopy. The most abundant nanoparticles identified in surface water were silica, titanium dioxide, and iron oxide with 4-99 nm diameter. The extraction efficiencies of CPE for silicon, titanium, and iron elements from environmental water samples were 51%, 15%, and 99%, respectively. This study applied CPE with TEM to enrich and analyze popular nanoparticles such as SiO₂ and TiO₂ from natural waters, which has not been well addressed by previous researches. Overall, CPE coupled with transmission electron microscopy (TEM) can be an effective method to characterize NMs in aqueous water samples, and further optimization will increase the extraction efficiency of NMs in complicated surface water matrix.

Keywords: nanomaterial, exposure, cloud-point extraction, water

1. Introduction

Engineered nanomaterials (ENMs) are widely used in industry and consumer products, which are defined as materials at least one dimension in the range of 1-100 nm (Meyer et al., 2009; Li and Chen, 2011; Maynard, 2011; Ripp and Henry Theodore, 2011). The daily use of consumer products containing ENMs [e.g., sunscreens with nano titanium dioxide (TiO_2) and foods with nano silica (SiO_2)] inevitably leads to human and environmental exposure to ENMs, which poses potential threat on human health and negatively impact environmental processes (Popov et al., 2005; Benn and Westerhoff, 2008; Reinhart et al., 2010; Klaine et al., 2012; Yang et al., 2013). Nanomaterials in consumer products can be released to sewer system, wastewater treatment plants, and surface water through washing and other processes (Benn and Westerhoff, 2008; Kiser et al., 2009; Keller et al., 2013). The composition and morphology of ENMs in surface waters is critical to understanding ENM transformations, fate, and toxicity. Currently, there is limited information on the concentration and morphology of ENMs in natural water, and many existing studies rely on artificially high ENM concentrations and lab-synthesized NMs (Scown et al., 2010; Mitrano et al., 2014; Zhang et al., 2016). Thus it is important to clarify the abundance and existence of NMs in natural waters.

Because ENM concentrations in surface water are on the order of 0.001 ng/L (fullerenes) to 2.2 $\mu\text{g/L}$ (nano TiO_2) using probabilistic modeling and measurement (Gottschalk et al., 2009; Gottschalk et al., 2013), concentrating NMs from water is required to have adequate masses for quantification and morphology analysis (Lowry et al., 2012; Hawthorne et al., 2014). Additionally, applying transmission electron microscopy (TEM) directly on natural water samples often found no NMs upon our analysis of more than 10 samples from canal and river in

Arizona area (details in SI). Thus pretreatment to enrich ENMs can benefit the subsequent mass and morphology analysis of NMs in natural water. Cloud-point extraction (CPE) using a surfactant is simple and noninvasive pretreatment that can be easily operated in a chemical lab (Wei et al., 2004; Liu et al., 2009b).

Generally, CPE uses a small volume of surfactant in a larger volume of water. The combined solution is heated above the cloud temperature of surfactant to form micelles of surfactant and targeted nanoparticles (Paleologos et al., 2005; Liu et al., 2009b; Samaddar and Sen, 2014). The mixture is then cooled to below the cloud temperature of surfactant, and a surfactant phase containing NMs separates from the water matrix, enriching targeted materials in the surfactant phase (Carabias-Martinez et al., 2000; Paleologos et al., 2005). CPE has a proven a high extraction efficiency of 82%-105% on nano Ag with Triton 114, regardless of capping agent (except bovine serum albumin), salts, organic matter, and inorganic colloids (Hartmann et al., 2014). CPE preferentially transfers silver nanoparticles rather than silver ions to the surfactant Triton 114 phase (Chao et al., 2011; Yu et al., 2013). Thus CPE can be an effective pretreatment to separate and concentrate NMs from natural waters before NM characterization by TEM or other spectroscopy techniques.

Most previous research focused on NM recovery from synthetic water or natural water with spiked ENMs. For instance, combined use of TX-114/TX-100 mixtures for CPE has been explored on Au and palladium (Pd) nanoparticle in synthetic water with extraction efficiency of 43%-52% (Nazar et al., 2011). TX-114 also demonstrates varied extraction efficiency of CPE

(59%-108%) in river water dosed with copper oxide nanoparticles (Majedi et al., 2014). However, only a few reports are available on using CPE for nano silver (Ag) and nano gold (Au) quantification in natural waters (Hartmann and Schuster, 2013; Li et al., 2013). and CPE coupled with mass spectroscopy was used to quantify nano Ag concentrations in the influent and effluent of nine WWTPs in Germany, which were 0.06 -1.5 µg/L and 0.001-0.012 µg/L, respectively (Li et al., 2013). However, application of CPE to enrich other popular ENMs (e.g., SiO₂ and TiO₂) from natural water is still limited. Thus it is necessary to validate the CPE technique coupled with TEM for NM enrichment and characterization (Duester et al., 2016).

The objective of this paper was to explore the extraction efficiency of CPE on a variety of ENMs and apply it with TEM for characterization of NMs from natural water. First, tests were conducted to quantify extraction efficiencies for nano-TiO₂, SiO₂, Ag, and Au. We focused on exploring impact of particle size and capping agent on CPE efficiency using the nano Au, since nano TiO₂ and SiO₂ explored in this study have fixed particle size range, while being widely used without capping agent (Yang et al., 2014a; Speed et al., 2015). Additionally, previous research have proven negligible impact of particle size (2-80 nm) and capping agent on CPE efficiency, except bovine serum albumin (BSA) stabilized nano silver (Liu et al., 2009; Hartmann et al., 2014). In order to check the presence and morphologies of NMs in natural water, CPE was then applied to enrich NMs from local surface waters, and materials enriched in the surfactant phase were characterized by TEM coupled with Energy Dispersive X-ray Spectroscopy (EDX). The concentration of elements in the surfactant and aqueous phases were analyzed after microwave digestion by inductively coupled plasma mass spectrometry (ICP-MS).

2. Materials and Methods

2.1. Sources and Characterization of Nanomaterials

Nanomaterial source, application, size, and capping agent for the NMs in this study are provided in Table 1. Nano Ag and nano Au were synthesized and stabilized with citric acid or polyvinylpyrrolidone (PVP, Sigma-Aldrich, MO, USA) following established protocols (Yang et al., 2012; Piella et al., 2016)). Briefly, 14 mM of silver nitrate (AgNO_3 , EM Science, NJ, USA) or gold (III) chloride (AuCl_3 , Sigma Aldrich, MO, USA) stock solution was introduced to citric acid (final concentration, 1% by weight) or PVP solution (final concentration 0.06% by weight). Sodium borohydride (20 mM, NaBH_4 , Sigma) was gradually dosed to solution upon 1.5 folds of amounts required to fully reduce Ag/Au ions to zero valent. Tannic acid stabilized nano Au was obtained from Nano Composix (CA, USA). Food-grade (E171) TiO_2 was obtained from a supplier in China and has been thoroughly characterized elsewhere (Yang et al., 2014a), 20% of particles (by number) were smaller than 100 nm in diameter. A TiO_2 catalytic material (P25) was obtained from Evonik Degussa Corporation (Vernon Hills, IL, USA) (Weir et al., 2012). Both food-grade TiO_2 and P25 suspensions were dispersed in 10 mM phosphate buffer (pH 7.8) and further sonicated by water bath sonication for 30 minutes before CPE test (Jassby et al., 2012). Colloidal nano SiO_2 particles used as chemical polishing (CMP) agent were obtained from Cabot Corporation. The nano SiO_2 particles had an average diameter of 38 nm, and a complete characterization is provided elsewhere (Speed et al., 2015). The hydrodynamic diameters were determined by phase analysis light scattering (ZetaPALS, Brookhaven Instruments Corporation, NY, USA) after adding 1 mg/L of nanoparticles to ultrapure water (Millipore Inc.) without pH adjustment.

2.2. CPE Procedure

All chemicals were obtained from Sigma-Aldrich (MO, USA) unless specifically states. We used the surfactant Triton X-114 (TX-114) for CPE. A modified CPE procedure that has a demonstrated high extraction efficiency was used for all tests (Hartmann et al., 2013). Firstly, nanomaterial suspension with an initial concentration of 1 mg/L was prepared in ultrapure water (details in SI information). After that, 40 mL of 1 mg /L was combined with 400 μ L of 1.25 M sodium acetate solution, 100 μ L of 1 M acetic acid, 1.0 mL of saturated EDTA solution, and 1.0 mL of a 10% TX-114 solution (w/w in water, (Hartmann et al., 2013; Hartmann et al., 2014). The suspension was mixed on a vortex in a polypropylene centrifuge tube (VWR, USA), incubated at 40 °C in a water bath for 30 min, centrifuged at 2,650 g for 12 min, and eventually cooled at 4 °C. The aqueous supernatant solution was pipetted out, and 0.5 mL of the surfactant phase remaining at the bottom of the tube was used for further study. The CPE extraction efficiency was defined as $M_I/M_T \times 100\%$, where M_I is the mass of NMs extracted in the surfactant phase and M_T is the total mass of NMs in the initial aqueous phase. The CPE enrichment factor was the ratio as the NM concentration extracted in the surfactant phase to the NM concentration in original water.

2.3. Effect of Nanomaterial Size and Matrix on CPE Extraction Efficiency

Three sizes of nano Au (stabilized by citric acid) at hydrodynamic diameters of 3.2 nm, 50 nm, and 87 nm were dispersed in nanopure water to a same concentration of 5 mg/L. After CPE, the enriched surfactant phase was re-suspended to 40 mL in methanol, sonicated in a water bath for 10 minutes, and analyzed by a UV-Vis spectrophotometer (DR 5000™, Hach, CO, USA). The impact of water matrix on CPE was studied using nanopure water and filtered wastewater from a

local wastewater treatment plant (WWTP) that employs activated sludge treatment and produces a well denitrified effluent after tertiary filtration.

2.4. CPE Extraction Efficiency for Nanomaterials in Nanopure Water

Prior to using CPE in natural water, a simple aquatic matrix (nanopure water) was used to determine CPE efficiency on a variety of NMs with different capping agents, including phosphate-stabilized TiO₂, SiO₂, PVP-stabilized nano Ag, and tannic acid-stabilized nano Au. Each NM stock suspension was spiked in nanopure water to achieve an initial NM concentration of 1 mg/L. The CPE procedure and extraction efficiency calculations described in section 2.2 were then applied.

2.5. CPE on Environmental Waters and TEM Analysis on Particles in Surfactant Phase

To examine NM presence and concentrations in environment waters, CPE was performed on waters from the Salt River, Verde River, Tempe Canal, influent and effluent from a potable water treatment plant (WTP), and influent and effluent from a WWTP. All the sampling sites were near metropolitan Phoenix, AZ, and detailed descriptions of each site can be found elsewhere (Barry, 2014) . To remove large sands and plant debris, all water samples were filtered through 1.2 µm cellulos filter before CPE extraction.

Particles enriched in surfactant phase from water samples were further analyzed by TEM/EDX. A 20-30 µL aliquot of surfactant phase sample was dropped on the top of a copper grid (TedPella, Inc., Ca, USA) coated with carbon film that was placed on clean KimWipe paper (Kimberly-Clark Professional, GA, USA). The KimWipe paper was used to remove surfactant through the copper grid. The TEM grid with sample was dried under a 100-W heat light to remove residual organic matters. A clean TEM grid dropped with pure Triton X-114 was used as a negative

control for potential contamination during the TEM grid preparation or handling. Images were collected on a TEM (STEM Phillips CM-200) coupled with EDX. The particle size in each TEM image was measured by Image J software (<http://imagej.nih.gov/ij/>).

2.6. Digestion of Surfactant Phase/Filtered Water Samples and ICP-MS Analysis

Both the element mass in the surfactant phase and filtered water samples (filtrate passing through 1.2 μm filter) were digested for concentration analysis. An aliquot of 0.4 mL each original water sample was used for digestion. To determine the element mass in the surfactant phase after CPE, 0.2 mL of surfactant phase was digested in a microwave oven at 900 w for 30 min, using 8 mL of 70% nitric acid (ULTREX® II Ultrapure Reagent, J.T. Baker, Avantor Materials, PA, U.S.), 4 mL of hydrochloric acid (33%–36% w/w, ULTREX® II Ultrapure Reagent, J.T. Baker, Avantor Materials, PA, U.S.), and 2 mL of hydrofluoric acid (47–51% w/w, ULTREX® II Ultrapure Reagent, J.T. Baker, Avantor Materials, PA, USA). . The digested solution was evaporated on a hot plate at 180 °C until between only 2-5 mL of solution remained. The remained solution was washed out with 5% nitric acid solution and further analyzed by ICP-MS (XSERIE-2, Thermo Scientific, MA, USA). The total mass of elements determined in the surfactant phase and filtered water (matrix before CPE) were normalized to the same original sample volumes as element concentration values.

Our previous work showed the presence of nano silica in the WWTP influent and effluent, thus the surfactant phase and original WWTP water samples were digested by 10 mL of tetramethylammonium hydroxide (TMAH, 25% in water, J.T. Baker, Avantor Materials, PA, USA). All other water samples were digested by nitric acid and hydrochloric acid and further

analyzed by ICP-MS. We analyzed approximately 40 elements including elements commonly associated with ENMs (Si, Au, Ag, Ti), as well as suite of other common elements as listed elsewhere (Yang et al., 2014b). Results presented here include elements with concentration higher than ICP-MS detection limit (1 ug/L).

2.6. Statistical Analysis

Differences in extraction efficiencies were determined by one-way ANOVA analysis using the software SPSS 11.5 for Windows (SPSS Inc., IL, USA). The normality of replicate data was examined when necessary upon Shapiro–Wilk's W test. Non-normal data was compared using Kruskal–Wallis non-parametric ANOVA. All the data involved in ANOVA analysis were tested for homogeneity of variances using Lavene's test. The significant level was assigned as 0.05 in all the tests.

3. Results and Discussion

3.1. Impact of Au Nanoparticle Size on CPE Extraction Efficiency

While impacts of solution pH, ionic strength, organic matter, and NM capping agents on extraction efficiency has been reported (Liu et al., 2009b; Hartmann et al., 2014), direct comparisons of CPE extraction efficiency as a function of NM size are not currently available. Figure 1 compares extraction efficiencies for citrated stabilized Au with diameters of 3.2 nm, 50 nm, and 87 nm. Extraction efficiencies are $95.8 \pm 5.9\%$, $100 \pm 2.4\%$, and $96.7 \pm 2.4\%$, respectively, and are statistically equivalent ($p = 0.35$). Other research showed high extraction efficiency of citrate-stabilized nano Au particles from 80–100%, while low extraction efficiency was observed on Au particle with diameter about 150 nm (Hartmann and Schuster, 2013).

Therefore, we concluded that the nanoparticle size of Au had negligible impact on CPE extraction efficiency.

3.2. Solution Matrix Effect on CPE Extraction Efficiency upon Au Nanoparticles

Comparable extraction efficiencies ($p = 0.198$) were obtained for nano Au stabilized with citric acid in nanopure water ($97 \pm 4\%$) and filtered WWTP effluent ($93 \pm 1\%$) (Figure S1). These extraction efficiencies were similar to those reported elsewhere for Au NMs in more saline water matrices (e.g., 18 mM NaCl) or water containing organic content (e.g., 30 mg/L humic acid) (Liu et al., 2009a; Liu et al., 2009b). CPE also demonstrated a high extraction efficiency (91–103%) for nano Au spiked in WWTP effluent (Hartmann and Schuster, 2013). Therefore, over the foreseen range of urban waters, we expected high and consistent NM extraction efficiencies..

3.3. CPE Extraction Efficiency for ENMs

Figure 2 shows the CPE extraction efficiency for five different ENMs. All the ENMs showed CPE efficiencies ranging from 83–107%, which were in the qualified range of 70–120 % for ecological effect test in EPA guidelines (USEPA, 2012). The extraction efficiencies for food-grade TiO_2 and P25 TiO_2 were $83 \pm 9\%$ and $96 \pm 13\%$ ($p = 0.38$), respectively. No significant differences existed in CPE efficiency between food-grade TiO_2 and P25 TiO_2 , despite their differences in phosphate content, zeta potential, and hydrodynamic diameters (Yang et al., 2014a). The extraction efficiency for nano SiO_2 , nano Au (tannic acid), and nano PVP-Ag were $107 \pm 5\%$, $79 \pm 8\%$, and $100 \pm 6\%$, respectively. Previous studies showed CPE for ENMs such as nano- Fe_3O_4 , Ag, and Au with extraction efficiencies ranging from 90%–97% (Liu et al., 2009b; Hartmann et al., 2014). Our findings were consistent with past work on individual NMs, and here we extended the application of CPE to other widely-used NMs (e.g., food-grade TiO_2

and SiO₂ used as polishing agents). Enrichment factor ranged from 66–86, proving the effectiveness of CPE to concentrate a wide spectrum of NMs.

The NM capping agent and surface charge affected CPE efficiency. For instance, the recovery of nano Au stabilized with tannic acid ($79 \pm 9 \%$) was lower than the nano Au stabilized with citric acid ($\geq 95\%$), which might be due to the weaker covalent bond of tannic acid with Triton X-114 (Paleologos et al., 2005). Suspensions of food-grade TiO₂, P25, and nano SiO₂ tested did not have capping agent, which were likely stabilized by static force (Badawy et al., 2010). Increasing of ionic strength during CPE could lead to their aggregation, which might trigger more interaction between particles and TX-114. CPE efficiency of less than 60% was observed for nano Ag without carbonate buffer (Hartmann et al., 2013), indicating that altering surface charge (or zeta potential) could affect the CPE efficiency. Overall, more research to explore interaction force between TX-114 and NMs was needed for clarifying the exact mechanism.

3.4 NM Morphology in Environmental Water

Figure 3 presents representative TEM images of ENMs extracted by CPE from environmental water samples. In the Salt River sample (Figure 3a), nanoscale colloids containing Ti and O were observed, and nanoscale particles with Fe, C, and O were found in the Verde River (Figure 3b). The aggregates of nanoparticles observed in the Salt River showed an average diameter of 60 ± 12 nm, and the EDX spectrum elemental ratio of Ti to O was 1:2, indicating they were likely TiO₂. It is unclear if the TiO₂ originated from recreational activities in this river stretch or from natural geological sources (Hem, 1985; Chiu and Westerhoff, 2010). NMs containing iron and carbon or oxygen having a maximum diameter of 93 nm were embedded in the residual

surfactant present in the CPE phase from the Verde River. These iron-containing particles might be either iron carbonate or iron hydroxide—both of which are common natural geological mineral forms. Thus, while CPE extracted NMs, it remained challenging to attribute their source to human/engineered versus natural or incidental sources.

The Tempe Canal conveys Salt and Verde River water to large WTPs in the Phoenix metropolitan area. In the Tempe Canal water, nano SiO₂-like particles (diameter: 46 nm, Figure 3c) were identified in the CPE phase using TEM/EDX. Figures 3d, 3e, and 3f show calcium carbonate particles (61 nm diameter), amorphous calcium silicate (120 nm diameter), and SiO₂ aggregates (22 nm diameter) in the WTP influent. Spherical SiO₂ NMs were identified in the WWTP effluent as well (Figure 3i and Figure S2), which were different sizes compared to SiO₂ in WWTP influent. These spherical silica-containing particles ranged in diameter from 43 to 220 nm. Silica NMs of these sizes are difficult to detect by other methods such as single particle ICP-MS (Lee et al., 2014). Even with fast scan improvements to sp-ICP-MS analysis to reduce background signals, sp-ICP-MS size limitations remained on silica particle above 100 nm at 1-10 µg/L (Lee et al., 2014; Montano et al., 2016). Thus CPE coupled with TEM/EDX might be a preferable way for quantifying silica NMs from waters compared to single particle ICP-MS.

Figure 3g shows iron oxide NM aggregates with an average length of 38 ± 8 nm, which could be from engineered food additives, incidental NMs from iron pipe corrosion, or other sources. In WWTP influent, aggregates of TiO₂ NMs with an average diameter of 30 ± 9 nm were observed (Figure 3h). Because TiO₂ has been widely used in personal-care products such as sunscreen and

toothpaste and because the particles showed similarity with morphology in raw powders (Yang et al., 2014a), these particles might have derived from food-grade TiO₂ sources. Separate work indicating other potential households sources of titania showed that washing textiles containing TiO₂ releases particles into the sewer that eventually reach the WWTP influent (Windler et al., 2012).

Figure 3 indicates that environmental waters (Arizona) contained titanium, silicon, iron, and calcium ENMs, and no silver or gold containing particles were identified in the 20 samples. Nano TiO₂ identified in water often existed as aggregates, which is consistent with previous observations in WWTP and biosolids samples (Westerhoff et al., 2011; Westerhoff et al., 2015). Individual and aggregates of primary amorphous (verified by HRTEM) nano SiO₂ particles were found. Nano iron oxides were typically part of aggregates. Identifying the NM sources is still a great challenge. Separate research has examined element ratios within individual nanoparticles (e.g., Ce/La ratios) to differentiate natural from engineered sources (Von der Kammer et al., 2012). However, there still lacked a common and reliable method to track other NMs such as silica. Further research is needed to track their anthropogenic/natural source.

3.5 Element Concentrations and CPE Efficiencies in Natural Waters

All samples were digested to determine the CPE extraction efficiency. In the original water filtrate, six elements (Na, Ca, Mg, Al, Si, and K) were present above the detection limit of 1 µg/L, and Ti concentrations were below the 1 µg/L detection limit. Na had the highest concentration in filtrate of water samples, ranging from 6.10–51.57 mg/L. The Ca, Mg, Al, Si, and K concentrations ranged from 0.46–16.64 mg/L. In surfactant phase after CPE, three

elements (Na, Ca, and Ti) were detected. The Na concentrations in surfactant phase were 7.7–10.5 mg/L, and the Ca concentrations in surfactant phase were 0.31–0.77 mg/L. We detected measurable Ti concentrations in surfactant phase (0.005 - 0.030 mg/L). Because the direct element analysis on water filtrate did not show a measurable Ti concentration, this indicated that enrichment by CPE was an effective method for concentrating elements and is necessary to analyze trace metallic colloidal particles in environmental water.

The CPE extraction efficiencies for all elements except Na and Ti were calculated by comparing element concentration in surfactant phase to that in filtrate of water. This was not possible for Na and Ti because salt addition interfered with measuring Na concentration in surfactant, and Ti concentration in water sample was zero. Mg, Al, Si, and K showed CPE efficiency of zero, indicating they were probably present as cationic ions. The CPE efficiencies for Ca ranged from 3 to 35%, with variation which might be attributed to the different water matrices.

3.6 Element Concentrations and CPE Efficiencies in Influent and Effluent of a WWTP

Figure 5a depicts the concentrations of all detectable elements in the surfactant phase from the influent and effluent of a WWTP (normalized the total sample volume of 40 mL). For water sample filtrates, eight elements (Na, Al, Si, Ca, Mg, Fe, Ti, and Ni) were present above the detection limit of 1 µg/L. Five elements (Na, Al, Si, Ca, and Mg) showed concentrations of 31.18–364.58 mg/L in WWTP influents and effluents. The influent Fe concentration (11.15 mg/L) was much higher than effluent Fe concentration (1.65 mg/L), indicating that most Fe was removed during the wastewater treatment process. Concentrations of Ti and Ni ranged from 0.30–2.46 mg/L. For surfactant phase after CPE, seven elements (Na, Al, Si, Ca, Mg, Fe, Ti)

were above the detection limit and ranged from 0.27–524.53 mg/L. The higher concentration of Na in surfactant was due to addition of sodium salts during the CPE process. Three elements (Al, Fe, and Ti) had measurable concentration in WWTP influent and were below detection limit in the WWTP effluent. The CPE efficiencies were also calculated for all elements except Na. The CPE efficiencies for Al, Si, Ca, Mg, Fe, Ti, and Ni in WWTP influent were 7%, 57%, 0, 94%, 99%, 15%, and 0, respectively. In WWTP effluent, the CPE efficiencies for Al, Si, Ca, Mg, Fe, Ti, and Ni were 0, 44%, 35%, 75%, 0, 0, and 7%, respectively.

3.7. Applying CPE/TEM for Nanomaterial Characterization

The combined application of CPE with TEM and ICP-MS provides a necessary and powerful tool to characterize and quantify NMs in water. Because NMs in water are estimated in the concentration range of 0.003–21 ng/L (Gottschalk et al., 2009) , it is almost impossible to characterize the morphology of NMs by TEM without the enrichment. CPE can concentrate NMs in a small volume of suspension, facilitating its application on a TEM stub for further analysis. CPE also preferentially enriches particulate metals and NMs to the surfactant phase through the micelle formation (Paleologos et al., 2005; Liu et al., 2009a). This reduces the interference from metal ions during the element composition analysis by EDX. Lastly, the pre-concentrating of elements is sometimes necessary to determine their concentrations (Li et al., 2013). In our case, when Ti concentration was around 1–10 µg/L in environmental water samples, it was difficult to quantify its concentration after TMAH digestion and further dilution (remove residual organic before ICP-MS analysis). However, the concentration calculated from the mass in the surfactant phase after CPE provided an estimate of element concentration.

4. Summary & Conclusion

We applied the CPE with TEM technique to NMs in nanopure water. CPE by Triton X-114 demonstrated the ability to enrich several ENMs, including nano Au, TiO₂, and SiO₂ with varied size and capping agent. Therefore, the approach of CPE by Triton X-114 was applied to enrich potential ENMs in environmental water and further characterize the NMs by TEM-EDX. The most abundant nanoparticles identified had diameters of 4–99 nm and contained Si and Ti. We further analyzed the element mass in the surfactant phase and calculated CPE efficiencies in environmental water. Overall, we found CPE coupled with TEM-EDX to be a powerful tool for enriching and characterizing ENMs in environmental water.

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**Prospecting nanomaterials in aqueous environments by cloud-point extraction coupled
with transmission electron microscopy**

Yu Yang^a, Robert Reed^a, Jared Schoepf^b, Kiril Hristovski^c, Pierre Herckes^d, Paul Westerhoff^{a*}

Affiliations:

^aSchool of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, Arizona, 85287-3005, United States

^bSchool for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, Arizona, 85287-6106, United States

^cThe Polytechnic School, Arizona State University, Mesa, Arizona, 85212, United States

^d School of Molecular Sciences, Arizona State University, Tempe, Arizona, 85287-1604, United States

***Corresponding Author and Address:**

Paul Westerhoff, School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, Arizona 85287-3005, United States

Phone: 480 965-2885

E-mail: p.westerhoff@asu.edu

Table 1. Summary of ENMs used in CPE study

ENMs	Source	Application	Primary particle size (nm) by TEM	Capping agent (stabilizer)
E171 TiO ₂	A supplier from China	Food additive	124 ± 42	None
P25 TiO ₂	Evonik Degussa, IL, USA	Catalyst	23 ± 10	None
Colloidal SiO ₂	Cabot Corp, MA, USA	Chemical mechanical polishing	38 ± 14	None
Nano Au-Tannic Acid	Nano Composix, CA, USA	Reference NM	50 ± 4	Tannic acid
Nano Ag- PVP	Lab synthesized	Reference NM	21 ± 8	PVP
Nano Au-Citric acid	Lab synthesized	Reference NM	3.2 ± 0.1 ^a	Citric acid
Nano Au-Citric acid	Lab synthesized	Reference NM	50 ± 1 ^a	Citric acid
Nano Au-Citric acid	Lab synthesized	Reference NM	87 ± 1 ^a	Citric acid

^a Size was measured by phase analysis light scattering (PALS).

Figure 1. The extraction efficiency of citrate-functional Au nanoparticles of different sizes. Prior to CPE, concentrated NM suspensions were diluted to 1 mg/L using nanopure water [pH 6.8, 0.055 $\mu\text{S}/\text{cm}$ (equal to 18.2 $\text{m}\Omega\cdot\text{cm}$)] without any additional buffer. Error bar represents data range of duplicate CPE experiments.

Figure 2. The extraction efficiency of five ENMs. Error bar represents two standard deviations of duplicate tests with triplicate measurement. Prior to CPE, concentrated NM suspensions were diluted to 1 mg/L using nanopure water [pH 6.8, 0.055 $\mu\text{S}/\text{cm}$ (equal to 18.2 $\text{m}\Omega\cdot\text{cm}$)] without any additional buffer.

Figure 3. Characterization by transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDX) on extracted NMs from environmental waters: (a) nano TiO_2 in Salt River, (b) iron carbonate-like nanoparticle in Verde River, (c) nano SiO_2 in Tempe Canal, (d) CaCO_3 -like nano particle in WTP influent, (e) CaO and SiO_2 -like nanoparticle in WTP influent, (f) amorphous nano SiO_2 in WWTP influent, (g) iron oxide nanoparticle in WWTP influent, (h) nano TiO_2 in WWTP influent, and (i) nano SiO_2 in WWTP effluent. The scale bar represents 100 nm in all the images except images a and c of 50 nm. The area with red circle/rectangular/arrow was where EDX was conducted, and the major elements detected by EDX are presented on each image. Images were selected from analysis on about 20 samples.

Figure 4. Metal concentrations in water samples: (a) upon direct digestion in filtered water samples and (b) upon digestion of surfactant phase after CPE from Salt River, Verde River, WTP influent and effluent, and a canal. The detection limit of ICP-MS for all elements was about 1 $\mu\text{g/L}$. BDL = below detection limit.

Figure 5. Measured element concentrations in (a) filtered water samples and (b) upon digestion on surfactant phase after CPE in WWTP influent and effluent. In order to measure silicon (Si) concentration, all samples were digested by TMAH.

**Cloud Point
Extraction**

TEM

NMs in Natural Water