Bulgarian Chemical Communications, Volume 50, Issue 2, (pp. 306 –311) 2018

Cloud point extraction combined with flame atomic absorption spectrometry for analysis of trace silver nanoparticles in environmental waters

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Received January 8, 2018, Revised April 10, 2018

A cloud-point extraction (CPE) method using non-ionic surfactant Tergitol TMN-6 (TMN-6) is developed for the extraction and pre-concentration of silver nanoparticles (AgNPs). Effective separation of ionic silver species and AgNPs is achieved by using sodium thiosulfate ($Na_2S_2O_3$) as a masking agent. Addition of 1M $(NH_4)_2SO_4$ enhanced the phase separation and thus increased the extraction efficiency of AgNPs. An enrichment factor of 57 was obtained with 1% (v/v) TMN-6, and the recoveries of AgNPs spiked into environmental samples were in the range of 96.7% to 108%. A low limit of detection (LOD) of 6.5 μg/L was achieved using flame atomic absorption spectrometry (FAAS) for quantification. The relative standard deviation (RSD) was 2.1% (n=9). This proposed method provides an efficient approach for the analysis and tracking of AgNPs in environmental water samples.

Key words: Silver nanoparticles, Cloud point extraction, Pre-concentration, Flame atomic absorption spectrometry, Tergitol TMN-6.

INTRODUCTION

Metallic nanoparticles are of high importance in current materials research, with potential applications in biomedicine, energy conversion, imaging, or as pigments [1,2]. Engineered nanomaterials (NMs) have already yielded a variety of commercially available products including cosmetics, antimicrobials, suntan lotions, paints, stain-resistant clothing and remediation products [3]. The rapid growth in the commercial use of AgNPs will inevitably increase silver exposure to the general population [4]. AgNPs may penetrate the skin [5] *via* textile or wound dressing contact, be inhaled from some spray medicines, or ingress into the female genital tract by incorporation from female hygienic products or medical implants [6]. It is also toxic to human cells [7-9]. Therefore, it is important from an analytical point of view to develop sensitive and economical methods for determination of trace silver nanoparticles.

A great deal of methods have been developed for characterization and quantitative analysis of NMs in simple matrices, as well as in natural NMs in a complex matrix such as environmental waters and soils [10,11]. In the case of AgNPs, the identification was usually conducted by the combination of scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), and UV-vis spectroscopy [12,13], whereas the quantification was performed after oxidation of AgNPs to Ag^+ and detection with inductively coupled plasma mass spectrometry (ICP-MS) [14,15], graphite furnace atomic absorption spectrometry [16,17], inductively coupled plasma optical emission spectrometry (ICP-OES)[18], or chromogenic methods [19-21]. Due to the low concentration of analyte and matrix effects, often a suitable sample pretreatment step (pre-concentration and separation) is required.

Cloud-point extraction (CPE), which is based on the fact that most nonionic surfactants in aqueous solutions form micelles and become turbid when heated to the cloud point temperature or in the presence of an electrolyte. Above the cloud point, the micellar solution separates into a surfactant-rich phase with a small volume and a diluted aqueous phase [22]. To date, CPE has been successfully applied for the quantification of Au-NPs in water samples in combination with electrothermal atomic absorption spectrometry (ET-AAS) [23]. However, in ET-AAS measurements, due to large background absorption signals and sample inhomogeneity, the relative sensitivity and analytical precision are poor.

The typical surfactants used in CPE processes mostly include Triton series, lgepal series, PONPE series, etc. [24,25]. However, these surfactants contain alkyl phenyl groups in their hydrophobic moiety, leading to some environmental concerns [26]. Therefore, biodegradable surfactants are proposed such as ethoxylated alcohols without phenyl group and branched alkyl chains. Tergitol

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series surfactants are polyethylene glycol trimethylnonyl ethers, non-ionic surfactants, with no aromatic group; with cloud-point temperature of 36℃, similar to that of Triton series. Therefore, the big potential of Tergitol TMN-6 in cloud point extraction will be taken advantage of.

In this study, the possibility of AgNPs enrichment by CPE based on TMN-6 as extractant was considered. A new microextraction method combined with flame atomic absorption spectrometry (FAAS) was developed for separation, enrichment and determination of AgNPs in environmental waters. Factors affecting the extraction efficiency, such as pH, concentration of extractant, extraction time, and nature of the extractant were studied and optimized.

EXPERIMENTAL

Chemicals and materials

All chemicals used were of analytical grade or better and all solutions were prepared in deionized water. Tannic acid, sodium carbonate, ammonium sulfate, hydrochloric acid, silver nitrate, ethanol, Tergitol TMN-6 and sodium thiosulphate, all were purchased from (Aladdin, China).

pH SJ-4A pH meter (Shanghai, China), vortex agitator (Shanghai, China), SK5210LHC ultrasonic instrument (Shanghai, China), centrifuge (Shanghai, China) and flame atomic absorption spectrometer (AA6300, Shimadzu, Japan) were used. Samples of waste water treatment plant (WWTP) were obtained from Chengong (Kunming, China), the river water sample was from Luo Long River (Kunming, China).

Nanoparticles synthesis

Preparation of $AgNO_3$ solution and tannic acid solution: 0.100 mol/L Na_2CO_3 solution in deionized water; 200 mL tannic acid solution were added to a three-mouth bottle in a thermostated water bath under stirring; 5mL of Na_2CO_3 solution was added for tannin reduction; after 10 min, a certain amount of $AgNO_3$ solution was dropwise added to the above mixture with a constant-pressure drop funnel. The color of the solution in the tree-mouth bottle gradually changed from colorless to light yellow, orange, red, and finally turned a deep red or red brown. The stirring and heating continued for 30 min more to obtain the AgNPs. The AgNPs concentration of the solution was determined by FAAS.

CPE of AgNPs

AgNPs sample solution (4 mL) was placed in a 10 mL long tapered glass centrifuge tube and pH was adjusted to 3.0 with dilute HCl. Then, 40 μL of 1M $Na_2S_2O_3$, 80 μL of 1% (v/v) TMN-6 and 40 μL of 1M $(NH_4)_2SO_4$ were added in sequence. The mixture was mixed and incubated at 36℃ in a water bath for 15 min, and the tube was centrifuged at 3000 rpm at room temperature for 15 min to facilitate phase separation. The obtained TMN-6-rich phase (<100μL), which forms a viscous droplet was attached to the bottom of the tube. The supernatant aqueous phase can then easily be removed by decanting. The droplet was dissolved in 2 mL of ethanol and was submitted to FAAS measurement.

RESULTS AND DISCUSSION

Optimization of the CPE conditions

Parameters that commonly affect the CPE efficiency, such as salt content, surfactant concentration, temperature, pH, and incubation time, were optimized. pH plays an important role on metal chelate formation and subsequent extraction. To optimize the extraction conditions for the nanoparticles pH was varied between 1 and 7 using hydrochloric acid for pH adjustment while the other experimental variables remained constant. The highest extraction efficiency was achieved at pH 3.0 (Fig. 1) (number of replicates n=6). So, pH 3.0 was selected as the optimum pH value for further experiments.

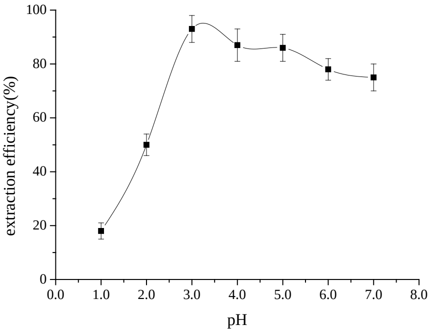

Fig. 1. Effect of pH on CPE of AgNPs

For the highest possible enrichment the final volume of the nanoparticle-rich phase should be as small as possible. On the other hand, the extraction efficiency of nanoparticles should not be decreased by the use of an insufficient amount of surfactant. Experiments with different amounts of TMN-6 at constant overall volume and nanoparticle concentration (Fig. 2) showed that for an aqueous sample of 4 mL 80μL of 1% TMN-6 solution are sufficient to meet the above criteria.

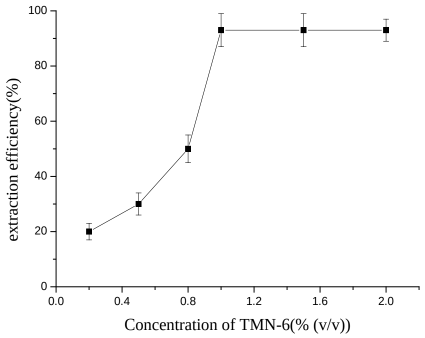

Fig. 2. Effect of TMN-6 concentration on the extraction efficiency

The CPE behavior was also affected by salt concentrations. Addition of salt can accelerate phase separation and lower the cloud point temperature of the surfactant solution. In this study, $(NH_4)_2SO_4$ was added as a salt to enhance the extraction of AgNPs, and $Na_2S_2O_3$ as a masking reagent to cover the interference of Ag^+. When $Na_2S_2O_3$ was added, thiosulfate ions and silver ions generated thiosulfate (Fig. 3), which is soluble in water. In this way silver ions can be eliminated prior to the detection of AgNPs.

$$Na_2S_2O_3+2Ag^+=2Na^++Ag_2S_2O_3\downarrow$$

$$Ag_2S_2O_3+2Na_2S_2O_3=Ag_2Na_4(S_2O_3)_3$$

Fig. 3. The reaction of sodium thiosulfate with Ag^+

Fig. 4 shows the impact of $(NH_4)_2SO_4$ concentration on the extraction efficiency of AgNPs. The extraction efficiency increased with $(NH_4)_2SO_4$ concentration up to 1M while the other experimental variables remained constant. This effect may be due to increasing interactions between the analyte and salt in the sample solution with increasing salt concentration, due to the salting out effect of $(NH_4)_2SO_4$, and then remained constant up to 3M.

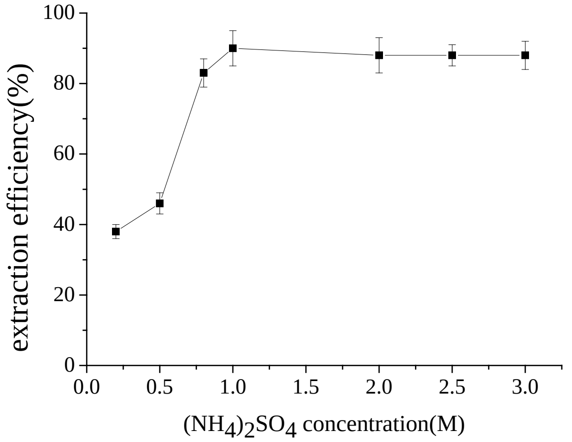

Fig. 4. Effect of $(NH_4)_2SO_4$ concentration on the extraction efficiency of AgNPs

To achieve easy phase separation, optimal equilibration temperature and incubation time are necessary to complete extraction. The effects of equilibration temperature and time were studied within the ranges of 30–50 ℃ and 15–40 min, respectively while the other experimental variables remained constant. It was found that an equilibration temperature of 36℃ and a time of 15 min were adequate to achieve quantitative extraction.

In this work, the kinds of surfactant such as Triton X-114, Triton X-100, TMN-6, TMN-10 and Tween 20 were investigated. The results are illustrated in Fig. 5. It shows that the extraction efficiency was the highest with the use of TMN-6 .

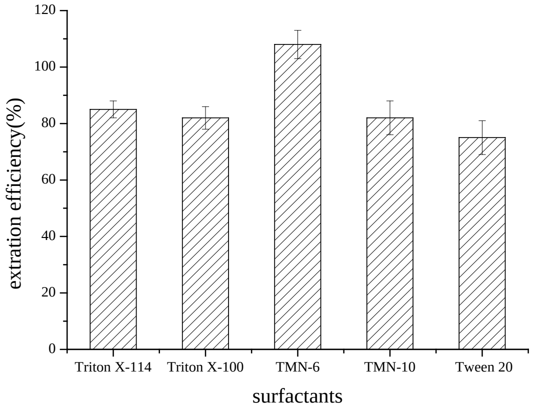

Fig. 5. Effect of surfactants on the extraction efficiency

The high viscosity of the so prepared surfactant-rich phase made it necessary to dissolve the sample prior to sample introduction to FAAS. Ethanol appeared to be a feasible solvent since it readily dissolves the surfactant-rich phase and prevents degradation of the enriched nanoparticles.

Evaluation of the preservation of the nanoparticle size distribution

To gain an insight in the size distribution of the nanoparticles in real samples it is necessary to know whether the size distribution of Ag-NPs is changing during the extraction procedure or not. This was studied using TEM for imaging of the particles before and after CPE. As shown, the size of the particles after the extraction process is more or less identical to the size before CPE treatment. Thereby we concluded that the size distribution is not affected by the present method. This supports the results of Yang *et al.* that studied CPE of metal nanoparticles [23]. This finding is particularly important because the size determination of nanoparticles is an important issue in the current research. CPE samples of Ag-NPs can therefore be used for size determination after pre-concentration.

The analytical characteristics of the method were evaluated under the optimum experimental conditions (Table 1).

Table 1. Analytical characteristics of the method

Parameter	Analytical feature
Linear range (μg/L)	10-1000
R^2	0.9999
Limit of detection (μg/L)	6.5
RSD(%) (n=9)	2.1
Enrichment factor	57
Pre-concentration time (min)	＜10
Recovery (%)	96.7-108.0

Analytical features

The enrichment factor (EF), relative standard deviation (RSD) and limit of detection (LOD) are effective factors to evaluate a developed analytical method. With the optimized system, the calibration graph of AgNPs was linear over the range of 10-1000 μg/L. The calibration equation is $A=7.9\times10^{-2}+0.003$ with a correlation coefficient of 0.9999, where A is the absorbance and C is the Ag concentration in μg/L. The detection limit, defined as the concentration equivalent to three times the standard deviation of the reagent blank for AgNPs was 6.5μg/L. The relative standard deviation for AgNPs was 2.1% (n = 9). The relative recoveries of AgNPs in water samples at the spiking level of 3-20 μg/L ranged from 96.7% to 108%. The enhancement factor, defined as the ratio of slope of pre-concentrated samples to that obtained without pre-concentration, was 57 for AgNPs.

Effect of foreign ions

With regard to the high sensitivity of FAAS determination of Ag, the interferences connected with the CPE procedure were studied due to the fact that foreign cations may react with TMN-6 and lead to a decrease in extraction efficiency. 10 mL sample solution containing Ag^+ and other ions was prepared and treated with the developed procedure. The tolerance limit was defined as the foreign ion concentration causing a change in the absorbance of less than ±5%. Table 2 shows the concentration of the interferent ions, the recovery is tolerable. No significant difference between recoveries for samples with or without filtration was observed, suggesting that the presence of natural particles in the samples had no significant effects on the CPE of AgNPs at a concentration of 20 μg/L. This result indicates that FAAS can be adopted to identify and quantify the AgNP content in environmental samples.

Analysis of real samples

To further evaluate the applicability of the proposed method, real environmental water samples were analyzed and the recoveries were determined by spiking 3-20 μg/L AgNPs. As shown in Table 3, the obtained recoveries of AgNPs were in the range of 96.7-108%, which was satisfactory with regard to the low spiking level.

Table 2. Effects of the matrix ions on the recoveries of the examined metal ions

Ions	Concentration (μg/L)	Recovery (%)	
		Pd	Ag
Na^+	10×10^3	105.0	104.1
K^+	10×10^3	104.2	104.8
Cl^-	10×10^3	103.5	102.2
Ca^{2+}	10	99.1	101.1
Mg^{2+}	0.5	99.2	96.8
Cr^{3+}	0.5	100.7	98.8
Mn^{2+}	0.5	103.4	99.2
Zn^{2+}	0.5	102.2	100.1
Cd^{2+}	0.5	98.7	99.7
Ni^{2+}	0.5	99.6	100.8
Pb^{2+}	0.5	108.7	98.5
Cu^{2+}	0.5	102.6	99.9

Table 3. Determination and identification of AgNPs in environmental waters by the proposed method

Sample	AgNPs added (μg/L)	AgNPs found (μg/L)	Recovery (%)
River water	0	*ND	—
	5	4.9±0.3	98.0±0.6
	10	10.8±0.5	108.0±5.0
	20	20.5±0.6	102.5±3.0
WWTP	0	*ND	—
	3	2.9±0.5	96.7±16.7
	8	8.1±0.6	101.3±7.5
	15	15.8±0.7	105.3±4.6

Table 4. Comparison of prposed measurement with other measurements

Measurement	Sample volume (mL)	Detection time (min)	RSD (%) (n=9)	Injection volume (mL)	LOD (µg/L)	Reference
ICP-MS	9.5	> 10	5.6	5	0.006	[18]
ET-AAS	40	5	9.5	0.2	0.005	[24]
FAAS	5	< 2	2.1	0.2	6.5	This paper

Comparison with other measurements

Table 4 compares the characteristic data of the proposed method with those of other methods for AgNPs determination reported in literature. As it can be seen, the proposed method offers a better RSD and a lower detection time.

CONCLUSION

In this report, CPE based on TMN-6 was demonstrated to be an efficient approach for selective extraction and pre-concentration of trace AgNPs from environmental water samples. $Na_2S_2O_3$ was added to the extraction system as a masking reagent of Ag^+ to prevent its extraction into the TMN-6 rich phase and $(NH_4)_2SO_4$ was added as a salt to enhance the extraction of AgNPs. The TMN-6-rich phase can be directly determined by FAAS in small sample volumes with a high sensitivity and good reproducibility. In addition, the type of sample and matrix does not affect the detection of AgNPs. This proposed method provided a simple approach for quantification of AgNPs in environmental water samples.

***Acknowledgements:** Haizhi Wu, postdoctoral researcher, being engaged in mineralogy and fluid geochemistry. The work was greatly supported by the Key Disciplines of Guizhou Province(ZDXK[2016]13), Medical Neurobiology Key Laboratory of Kunming University of Science and Technology, the Analysis and Testing Foundation of Kunming University of Science and Technology (No. 2017121).*

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ЕКСТРАКЦИЯ ПРИ ТОЧКАТА НА ПОМЪТНЯВАНЕ В СЪЧЕТАНИЕ С АТОМНОАБСОРБЦИОННА СПЕКТРОМЕТРИЯ ЗА АНАЛИЗ НА СЛЕДОВИ КОЛИЧЕСТВА ОТ СРЕБЪРНИ НАНОЧАСТИЦИ В ОТПАДНИ И ПРИРОДНИ ВОДИ

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Постъпила на 8 януари 2018 г.; коригирана на 10 април 2018 г.

(Резюме)

Разработен е метод за екстракция при точката на помътняване (CPE) с използване на нейонното повърхностно активно вещество тергитол TMN-6 за извличане и концентриране на сребърни наночастици (AgNPs). Ефективно разделяне на йонните форми на среброто от AgNPs е постигнато с помощта на натриев тиосулфат ($Na_2S_2O_3$) като маскиращ реагент. Добавянето на 1M $(NH_4)_2SO_4$ подобрява разделянето на фазите и повишава степента на екстракция на AgNPs. Фактор на обогатяване от 57 е постигнат с 1% (v/v) TMN-6, като извличането на AgNPs, добавени към водите, е в границите от 96.7% до 108%. Границата на откриване (LOD) с пламъков атомноабсорбционен метод за количествено определяне е 6.5 μg/L. Относителното стандартно отклонение (RSD) е 2.1% (n=9). Разработеният метод предлага ефективен подход за определяне и проследяване съдържанието на AgNPs в природни води.