Journal of Chromatography A, 1216 (2009) 5962–5967

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Size-fractionation of silver nanoparticles using ion-pair extraction in a counter-current chromatograph

Ching-Wei Shen, Tiing Yu*

Department of Applied Chemistry, National Chiao Tung University, Hsinchu 30050, Taiwan

ARTICLE INFO

Article history:
Received 25 January 2009
Received in revised form 28 May 2009
Accepted 2 June 2009
Available online 6 June 2009

Keywords:
Counter-current chromatography
Silver nanoparticles
Size separation
Ion-pair
Phase transfer
Step-gradient extraction

ABSTRACT

Size separation of silver nanoparticles was investigated in counter-current chromatography (CCC) based on a unique step-gradient extraction process. Carboxylate anions were modified on silver nanoparticles to produce water-dispersible nanoparticles. The aqueous nanoparticles were readily transferred to the organic phase (toluene/hexane = 1:1, v/v) together with the phase transfer catalyst, tetraoctylammonium bromide (TOAB), owing to the ion-pair adduct formation between silver nanoparticle anions and tetraoctylammonium cations. Smaller nanoparticles were found to be more readily transferred to the organic phase compared to larger nanoparticles. Various concentrations of TOAB in the organic elution phase were used in the CCC extraction experiments. It appeared that a concentration of 0.02 mM of TOAB was adequate to achieve optimum separation and recovery for the aqueous Ag nanoparticle sample (1.5 mg) in the CCC extraction experiments. Samples of 15.8 ± 5.3 nm were separated; the distributions of four fractions collected were 13.7 ± 1.9, 14.1 ± 3.5, 19.2 ± 4.3, and 22.2 ± 4.9 nm. Compared with the stepwise extraction performed in this study, the step-gradient extractions using CCC provided much better size discrimination.

1. Introduction

In the last decade, many separation techniques, including size exclusion chromatography [1,2], ion-pair chromatography [3], field-flow fractionation [4], gel electrophoresis [5,6], and diafiltration [7], have been developed for the size separation of nanoparticles. Ito et al. [8,9] demonstrated particle and cell separations using counter-current chromatography (CCC) in a non-synchronous coil planet centrifuge. Moreover, Fedotov [10] successfully fractionated micro-size particles suspended in single phase CCC using J type synchronous coil planet centrifuge. However, size separation of nanoparticles has not been reported using CCC. CCC is known as a support-free liquid–liquid chromatography [11,12], where irreversible adsorption would not occur. Accordingly, CCC is often applied in purifying crude extracts of natural products before regular HPLC/MS analysis [13,14]. In a typical CCC separation, a solvent system is made by mixing two or more solvents in a separatory funnel. After settling, two separate liquid layers (the upper and the lower phases) are formed. One of the two layers acts as the stationary phase and the other one is the mobile phase. Sample molecules are distributed in these two phases and separated during the elution. Typically, one of the phases is rich in water, i.e. hydrophilic; and the other one is rich in organic solvent, i.e. hydrophobic. However, water-dispersed nanoparticles are insoluble in hydrophobic media; thus, modifications of the solvent system are needed to adjust the partition between the two phases.

Hydrophobic surfactant, such as bis(2-ethylhexyl) sulfosuccinate (AOT) has often been applied as the stabilizing agent in the synthesis of metal nanoparticles in organic solvents [15,16]. Reverse micelles formed by AOT aggregates provide hydrophilic environments for nanoparticles. In addition, surfactant tetraoctylammonium bromide (TOAB) is a common cationic agent for phase transfer in nanotechnology. For example, $HAuCl_4$ can be transferred from water to toluene in the presence of TOAB and reduced to yield gold nanoparticles also stabilized by the surrounding TOAB molecules in toluene [17]. In addition to being a transfer agent for precursors and a stabilizing agent for nanoparticles, TOAB demonstrated extraction ability for noble metal nanoparticles that were modified by carboxyl groups possessing highly negative charges. The surfaces of gold or silver nanoparticles were usually capped by a monolayer of thiol molecules with Au–S, or Ag–S covalent binding. Thiol compounds, such as 11-mercaptoundecanoic acid (MUA) and mercaptosuccinic acid, were easily immobilized on gold and silver nanoparticles [18,19]. The extraction was attained through ion-pair formation between tetraoctylammonium cations (TOA^+) and the carboxyl group on nanoparticle surfaces [20]. Without TOAB, nanoparticles in the aqueous solution would not be transferred to toluene. With a low concentration of TOAB, relatively small-sized particles in the aqueous solution could be transferred more easily to the toluene. After the amount of small-sized particles

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* Corresponding author. Tel.: +886 3 573 1673; fax: +886 3 572 3764.
E-mail address: tyu@faculty.nctu.edu.tw (T. Yu).

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doi:10.1016/j.chroma.2009.06.002

decreased owing to the first extraction, more relatively large-sized particles would readily move to the organic layer during the second extraction. Therefore, size separation could be performed by stepwise extractions using the organic solvents with the same TOAB concentration. In addition to the concentration of phase transfer agent, organic solvents used as the extraction media played an important role on the phase transfer. Chloroform and carbon tetrachloride exhibited higher extraction efficiency for larger particles than toluene [21]. Thus, size-selective extraction of silver nanoparticles could be achieved using a series of different organic solvents containing the same surfactant.

In addition to chromatographic separations, CCC was used as a continuous extractor, for example, in removing surfactants from waste water [22]. The scale-up of dynamic extraction has been demonstrated and improved through the modification of CCC centrifuges [23]. The phase distribution in a CCC column used as a continuous extraction vessel has been studied recently in order to enhance extraction efficiency [24]. In the present study, size-separation of Ag nanoparticles using CCC was performed with solvent systems containing TOAB. Solutes dissolved in the aqueous stationary phase were eluted with the organic mobile phase. Although the extraction signal monitored using an on-line detector looked a little similar to chromatograms, the separation was achieved through a batch extraction process of Ag nanoparticles using a step-gradient of TOAB in the mobile phase.

2. Experimental

2.1. Reagents and solvents

HPLC-grade *n*-hexane and toluene were purchased from Tedia (Fairfield, OH, USA). Sodium borohydride, TOAB, and MUA were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Silver nitrate, hydrochloric acid, potassium dihydrogenphosphate (KH_2PO_4), and dipotassium phosphate (K_2HPO_4) were obtained from Showa (Tokyo, Japan). Water was purified using a Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.2. Preparation of aqueous Ag nanoparticles

Silver nanoparticles modified by MUA (Ag-MUA) were prepared using the method similar to the steps reported in the literature [18]. At the beginning, 1.7 mg of MUA was added into 25 ml of freshly prepared sodium borohydride aqueous solution (20 mM). After stirring for 5 min, 25 ml of silver nitrate solution (5 mM) was added by drops into the aqueous solution with vigorous stirring. The color of the clear solution turned to light yellow at first and finally to dark yellow due to the formation of silver nanoparticles. After 24 h of stirring, the solution was adjusted to pH 2 by adding 0.1 M HCl and the nanoparticles was precipitated by a centrifuge (Hermle Z323K centrifuge, Wehingen, Germany) running under 6000 rpm for 10 min. The precipitate (15 mg) was re-dispersed in 50 ml of phosphate buffer (20 mM, pH 11) as the stock solution. Because aggregates of silver nanoparticles would be enhanced by higher salt concentration [25], the phosphate buffer used in this experiment should be limited to 20 mM.

2.3. Analysis of Ag nanoparticles

2.3.1. Optical spectroscopy

The Ag-MUA stock solution (0.30 mg/ml) was diluted 30 times (owing to the high absorptivity) with deionized water to be characterized using a UV–vis spectrophotometer (Hewlett-Packard 8453, Waldbronn, Germany). In addition to the measurement of Ag-MUA in the aqueous solution, Ag-MUA in an organic solvent was also investigated. Phase transfer was also operated by mixing 10 ml of the stock solution with 10 ml of 1 mM TOAB-containing toluene/hexane (1:1, v/v) solution for 20 min. The upper organic phase was diluted 15 times with the hexane/toluene mixture and was measured with the UV–vis spectrophotometer.

To identify the functional groups on the surface of nanoparticles, a KBr pellet of the Ag-MUA powder dried under vacuum was made for measuring IR spectrum using a Bio-Rad FTS 165 infrared spectrophotometer (Philadelphia, PA, USA). A KBr pellet of the MUA powder was made to provide a blank measurement.

2.3.2. Electron microscopy

The synthesized Ag-MUA was diluted 100-fold by water and a small drop was tipped on a glass substrate and dried under vacuum. The substrate was sputtered to form a 2 nm platinum thin film in order to enhance the image resolution. The diameters of Ag-MUA were measured by a JEOL JSM-7401F (Tokyo, Japan) field emission scanning electron microscopy (SEM) system. The collected Ag-MUA nanoparticles from stepwise extraction and the CCC separations were also examined using the same instrument. In addition, the energy dispersive X-ray (EDX) spectrum was measured for the elements C, O, Ag, and S at the acceleration voltage of 10 keV.

2.4. Batch extraction using CCC instrument

The chromatograph used was a Model CCC-1000 (Pharma-Tech Research, MD, USA) high-speed CCC that was mounted in a temperature-controlled oven. It contained three spool-shape column holders; only one holder was used which was coiled with a 19-m long, 1.6-mm I.D. polytetrafluoroethylene (PTFE) tubing, with a total volume of 38 ml.

The coil was first filled with hexane/toluene (1:1, v/v) mobile phase solution without TOAB. The Ag-MUA stock solution prepared for the analytical purpose was also used as the sample solution for separation. The sample solution dispersed in phosphate buffer (20 mM, pH 11) was injected through a six-port injection valve with a coil of 5 ml into the tail end of the column. The rotation of the chromatograph was set at 700 rpm and the oven temperature was controlled at 20 °C. After sample loading, the extraction solution (TOAB-containing organic phase) was then pumped in using the tail-to-head mode. Flow rate was 1 ml/min for all experiments. The mobile phases were mixtures of hexane/toluene (1:1, v/v) containing various concentrations (0.1, 0.03, 0.02, and 0.01 mM) of TOAB. The effluent was monitored using a Bio-Rad Model 1801UV detector (Hercules, CA, USA) at 427 nm, and every 2 ml was collected using an Advantec CHF 121SA fraction collector (Tokyo, Japan).

2.5. Stepwise extraction of Ag-MUA nanoparticles

The aqueous solution of 1.5 mg Ag-MUA dispersed in 5 ml phosphate buffer (20 mM, pH 11) was mixed with the organic solution of 10 ml TOAB (0.02 mM)-containing hexane/toluene (1:1, v/v). After vigorous stirring for 20 min, the upper phase was removed and another fresh 10 ml organic phase was added for the second extraction. The process was repeated four times and the extracted portions were analyzed by SEM for the size distribution.

3. Results and discussion

3.1. Characterizations of Ag-MUA nanoparticles

The UV–vis spectra of the silver nanoparticles are shown in Fig. 1. The Ag-MUA nanoparticles in aqueous solution gave a maximum wavelength of surface plasmon resonance absorbance at 416 nm. In the absence of TOAB, mixing of the Ag-MUA aqueous phase with the hexane/toluene phase did not result in the successful transfer of