การแยกไอออนเงินและอนุภาคเงินระดับนาโนเมตรโดยใช้วิธีการสกัดแบบจุดขุ่นสำหรับตรวจวัดด้วยเฟลมอะตอมมิกแอบซอร์พชันสเปกโทรเมตรี

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SEPARATION OF SILVER IONS AND SILVER NANOPARTICLES USING CLOUD POINT EXTRACTION FOR THE DETERMINATION BY FLAME ATOMIC ABSORPTION SPECTROMETRY

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A Thesis Submitted in Partial Fulfillment of the Requirements
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ในงานวิจัยนี้ได้นำเสนอวิธีการแยกและวิเคราะห์หาปริมาณอนุภาคซิลเวอร์ระดับนาโนเมตร (AgNPs) และปริมาณไอออนซิลเวอร์ทั้งหมดด้วยการสกัดแบบจุดขุ่น (CPE) โดยใช้สารลดแรงตึงผิวชนิด Triton X-114 และเทคนิคเฟลมอะตอมมิกแอบซอร์พชันสเปกโทรเมตรี การแยก AgNPs ออกจากไอออนซิลเวอร์ (Ag^+) ทำได้โดยการเติมสารละลายโซเดียมไธโอซัลเฟต ($Na_2S_2O_3$) และ AgNPs ที่สกัดได้ในชั้นสารลดแรงตึงผิวจะมีความเข้มข้นเพิ่มขึ้น จากนั้นนำไปย่อยด้วยไฮโดรเจนเปอร์ออกไซด์ ก่อนการวิเคราะห์ปริมาณด้วย FAAS ในการสกัด AgNPs จะใช้สารละลาย $Na_2S_2O_3$ ความเข้มข้น 20 มิลลิโมลาร์ ที่ pH 3 โดยมีร้อยละการคืนกลับของ AgNPs อยู่ในช่วง 86.74-87.95% มีขีดจำกัดต่ำสุดของการตรวจวัด AgNPs เท่ากับ 0.27 มิลลิกรัมต่อลิตร ในการหาปริมาณทั้งหมดของซิลเวอร์ จะทำโดยออกซิไดซ์ AgNPs ในสารละลายให้เป็น Ag^+ จากนั้นเติม L-cysteine เพื่อให้เกิดเป็นสารประกอบเชิงซ้อนที่ไม่มีประจุกับ Ag^+ ก่อนการทำการสกัดแบบจุดขุ่น โดยร้อยละการคืนกลับของปริมาณซิลเวอร์ทั้งหมดจะอยู่ในช่วง 93.31-95.76% เมื่อใช้ L-cysteine 20 มิลลิโมลาร์ ในการวิเคราะห์สารละลายที่ปริมาณซิลเวอร์ทั้งหมด 0.5-1.0 มิลลิกรัมต่อลิตร ที่ค่า pH ในช่วง 1-5 ขีดจำกัดต่ำสุดของการตรวจวัดปริมาณซิลเวอร์ทั้งหมดเท่ากับ 0.11 มิลลิกรัมต่อลิตร

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PORNPIMOL KLOYSUWAN: SEPARATION OF SILVER IONS AND SILVER NANOPARTICLES USING CLOUD POINT EXTRACTION FOR THE DETERMINATION BY FLAME ATOMIC ABSORPTION SPECTROMETRY. ADVISOR: ASST.PROF. FUANGFA UNOB, Ph.D. 39 pp.

In this research, a method for separation and determination of silver nanoparticles (AgNPs) and total amount of silver was proposed by using Triton X-114 based cloud point extraction (CPE) and flame atomic absorption spectrometry (FAAS). The separation of AgNPs from ionic silver species (Ag^+) was achieved by addition of sodium thiosulfate solution ($Na_2S_2O_3$). The extracted AgNPs was concentrated into the surfactant phase and digested with H_2O_2 prior to the analysis by FAAS. The extraction of AgNPs from samples was performed by using 20 mM of $Na_2S_2O_3$ at pH 3.The recovery of AgNPs was in a range of 86.74-87.95 %. The limit of detection (LOD) of AgNPs was 0.27 mg L^{-1}. The total amount of silver was also determined by oxidizing AgNPs in solution to Ag^+. L-cysteine was added to form a neutral complex with silver ions before CPE was performed. The recovery of total amount of silver was in a range of 93.31-95.76% when 20 mM L-cystein solution was used in the analysis of solutions containing 0.5-1.0 mg L^{-1} of total Ag at pH 1-5. The limit of detection (LOD) of the total amount of silver was 0.11 mg L^{-1}.

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LIST OF ABBREVIATIONS

nm	Nanometer
µg	Microgram
$mg.L^{-1}$	Microgram per liter
$mg.L^{-1}$	Milligram per liter
^{o}C	Degree celsius
Transmission electron microscopy	TEM
AgNPs	Silver nanoparticles
ISE	Ion selective electrode
CPE	Cloud point extraction

CHAPTER I

INTRODUCTION

1.1 Statement of purpose

In recent year nanotechnology is growing rapidly. Nanoproducts are used in various fields. Silver nanoparticles (AgNPs) are increasingly found in consumer products such as food packaging, odor-resistant textiles, appliances in home and medical devices [1-3]. Silver nanoparticles show good antimicrobial properties due to its small size and large surface area. As a result, it provides high effectiveness for antimicrobial. It is suggested that silver nanoparticles penetrate into the cell wall of bacteria and associate with protein molecules [4]. More importantly, there is a potential application of AgNPs in the treatment of diseases that require maintenance of circulating drug concentration or targeting of specific cells or organs [5-6]. For example, AgNPs have been shown to interact with the HIV-1 virus and inhibit its ability to bind host cells in vitro [7]. At very low and reasonable concentrations, silver nanoparticles have no side effects on human health. Although it has potential antimicrobial activity, contact to silver ion for a long period causes diseases like argyria and argyrosis [8]. It causes adverse effects not only on human health but also on the environment such as in aquatic systems and soil microbial communities [9-11]. Until now the mechanism of toxic properties of silver nanoparticles has not been clearly explained. It is believed that the toxicity of silver nonoparticles is related to its release of Ag(I) ion, thus the determination of silver nanoparticles and silver ions in environmental samples are important[12-13].

To our knowledge, only few validated methods for the determination of nanoparticles are reported [14]. Because of the low concentration and the complex matrix of environmental and biological samples, not only a separation step but also the preconcentration step are required. The methods for the separation and the analysis of silver nanoparticle (AgNPs) have been proposed including cloud point extraction (CPE), field-flow fractionation (FFF), chromatographic method e.g.

hydrodynamic chromatography (HDC), counter-current chromatography (CCC) and size-exclusion chromatography (SEC). The most useful techniques for identification and characterization of AgNPs are transmission electron microscopy (TEM) and scanning electron microscopy (SEM). UV-Vis is another technique for silver nanoparticles characterization due to its optical properties. The techniques for determination of metal ions such as ICP-AES, ICP-MS, AAS and silver selective electrode can also be used but AgNPs samples have to be digested and oxidized before the analysis [15-16]. Although there are many techniques for characterization and determination of AgNPs at very low concentraion, to perform speciation, these techniques need to be coupled with separation method.

Cloud point extraction is an attractive method for separation and preconcentration of AgNPs. The technique is based on the property of non-ionic surfactants in aqueous solutions to form micelles and solution becomes turbid when it is heated up to the cloud point temperature. Surfactant can be separated from aqueous solution afterward. Under suitable condition, analytes are extracted and concentrated into surfactant rich phase for further analysis. Nowadays, cloud point extraction method has been used for the extraction and preconcentration of metal after formation of soluble complex with appropriate ligand [17].

In this study, sodium thiosulphate ($Na_2S_2O_3$) was used as a complexing agent to allow the separation between AgNPs and silver ions during cloud point extraction, due to the formation of a positively charged complex with silver ions. The extracted AgNPs was concentrated into the surfactant rich phase and then, was digested and analyzed by FAAS. To determine the total amount of silver, AgNPs were oxidized to silver ions prior to cloud point extraction. L-cysteine is chosen as chelating ligand to form complex with Ag^+ and enhance the extraction of silver into surfactant rich phase. The total Ag concentration in the surfactant phase was also determined by FAAS.

1.2 Objectives

1. To develop a method for separation and determination of silver nanoparticles and total amount of silver in solution by FAAS.
2. To apply the developed method to the analysis of real samples.

1.3 Scopes of this research

The methodology for separation and preconcentration of silver nanoparticles and silver ions by cloud point extraction was developed. For AgNPs determination, sodium thiosulphate ($Na_2S_2O_3$) was used as a complexing agent to mask silver ions. The amount of AgNPs was determined by FAAS after being digested with H_2O_2. L-Cysteine was used as a complexing agent to form complex with Ag^+ for total amount of Ag determination by FAAS after cloud point extraction. UV-Visible spectrophotometry and transmission electron microscopy (TEM) were used to identify and characterize AgNPs before and after extraction into the surfactant rich phase. Various parameters including concentration of $Na_2S_2O_3$, solution pH, size of nanoparticles, concentration of L-cysteine, co-existing ions were investigated. Finally, the method was validated and applied to real sample analysis.

1.4 The benefit of this research

To obtain a method for speciation analysis of AgNPs and total amount of silver based on cloud point extraction and determination by FAAS.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Silver nanoparticles

Silver nanoparticles are clusters of silver atoms with the size in the range of 1-100 nanometers. The small size of silver nanoparticles results in unique properties compared to the bulk silver. Due to their extremely large surface area to volume ratio, they have beneficial properties that are suitable for a wide range of application. Silver nanoparticles have been used in many commercial products such as optical sensors, electrical conductor and antibacterial agents. In addition to their most benefit antimicrobial effect, silver nanoparticles could cause toxic effect to human health. It can enter the body through various ways. There was also research reporting that AgNPs could release Ag^+ and the antibacterial activity is dominated by these ions [18]. Levard et al.[12] proposed that the toxicity of AgNPs is the result of the interaction of Ag ion with thiol groups of enzymes and proteins that affects cellular respiration and transport of ions across membranes and eventually causes cell death.

2.2 Chemical preparation of silver nanoparticles

Silver nanoparticles can be synthesized using various methods including chemical reduction, electrochemical reduction, γ-radiation, photochemical reaction and laser ablation [19]. Chemical reduction is the most popular method for preparing silver nanoparticles. Silver salt solution (e.g. $AgNO_3$) is used as starting reagent that is further reduced by reducing agent to produce colloidal silver. The reducing agents that have been commonly used include sodium citrate [20-22], sodium borohydride and ascorbic acid. Stabilizing agents are generally added into the silver salt solution in order to cap the particles and prevent the particle growth and particle aggregation. Certain polymers and organic molecules that can be attached on particles surface

would act as stabilizers. Therefore, the particle size and shape can be controlled by the choice of stabilizer used [19].

Citrate reduction method [20]

In this method, citrate ions play both the role of reducing agent and stabilizer. Silver nanoparticles obtained by this method have relatively large particle size (60 – 200 nm), compared to the particles obtained by using stronger reducing agents like sodium borohydride. Many researchers have demonstrated that these particles are useful in surface-enhanced Raman spectroscopic measurements [23-24]. However, the exact role of citrate ions in particle size controlling has not been clarified yet.

Mechanism of reaction could be expressed as follows.

$$4Ag^{+} + Na_3C_6H_5O_7 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow$$

2.3 Cloud point extraction method

Cloud point extraction (CPE) is a method for separation and preconcentration of target analytes from sample matrix [11]. It improves the detectability of the method and it is useful in trace element analysis. In CPE, a surfactant solution having concentration of its critical micelle concentration (CMC) is mixed with sample solution. When the mixture is heated until the cloud point of surfactant is reached, the surfactant micelles aggregates and they are no longer soluble. The solution becomes turbid and the surfactant phase separates from aqueous solution in small volume. The target analytes (e.g. organic compounds, metal ions) are extracted to the surfactant phase that is separated from aqueous phase for further analysis.

The extraction of analytes in sample occurs through the partition of the analytes to surfactant micelles [25]. This step is called “solubilization”. When the solution is heated to the cloud point of surfactant, the surfactant phase separates from aqueous sample and this step is called “phase separation”.

In metal ions analysis, chelating ligands are often added into the system to form hydrophobic complexes that can be extracted into surfactant phase. The main

limitation of CPE in metal ions extraction is the low partition coefficients of several metal ions or metal complexes. To overcome this drawback, highly hydrophobic ligands are often used.

An advantage of CPE over other extraction techniques such as solid phase extraction, liquid-liquid extraction and co-precipitation is the high preconcentration factor that can be achieved starting from small volume of sample. The metal will be preconcentrated in a small volume of surfactant phase (0.2 -0.5 mL). Furthermore, surfactant phase can be directly submitted to further metal determination techniques (e.g. spectroscopic techniques). On the other hand, the other techniques often require additional step of metal elution/re-extraction before submitting to an analytical instrument.

CPE is considered as green analytical method in respect to the perspective of "green chemistry". In CPE, non toxic, non volatile and non easily flammable surfactant is used instead of organic solvent. Due to the use of diluted solution of surfactant, the procedure is economic and generates small volume of waste.

Properties of surfactant and micellar formation

Surfactant molecules compose of a long hydrophobic hydrocarbon chain (R) and a small polar or ionic group (X). The hydrocarbon chain may contain 8 to 18 carbon atoms. Surfactants are commonly classified based on their hydrophilic group properties into four groups; cationic, anionic, amphoteric (or zwitterionic) and non-ionic (Table 1.1).

Table 1.1 Type and characteristics of surfactants

Type of surfactants	Characteristics
Anionic	The hydrophilic group carrying a negative charge such as carboxylate ($RCOO^-$), sulfonate (RSO_3^-), or sulfate ($ROSO_3^-$) Example : $CH_3(CH_2)_{11}SO_4^-Na^+$ Sodium dodecil sulfate (SDS)

Table 1.1 Type and characteristics of surfactants (continued)

Type of surfactants	Characteristics
Cationic	The hydrophilic group carries a positive charge such as the quaternary ammonium halides ($R_4N^+Cl^-$) Example : $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$ Cetyl trimetyl ammonium bromide (CTAB)
Non-ionic	The hydrophilic group has no charge such as polyoxyethylene ($-OCH_2CH_2O-$) or polyol groups Example : $CH_3(CH_2)_{11}(OCH_2(CH_2)_{23}OH$ Polyoxyethylene (23) dodecanol (Brij 35)
Amphoteric or zwitterionic	Its molecules contain both the anionic and cationic groups. Example : $CH_3(CH_2)_{11}N^+(CH_3)_2(CH_3)COO^-$ 4-(Dodeccyldimetyl ammonium) butirate (DAB)

When the concentration of surfactant reaches the critical micelle concentration (CMC), the surfactant molecules aggregate to form micelles (Figure 1.1). The CMC of a surfactant depends on its molecular structure and experimental conditions such as temperature, solution ionic strength and counter ions.

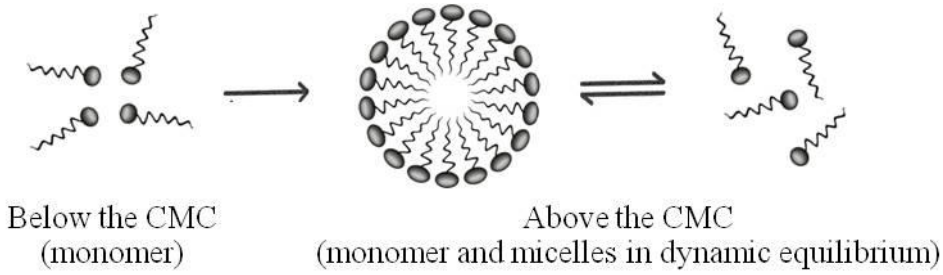


Figure 1.1 The aggregate micellar formation.

In CPE, non-ionic surfactants (e.g. polyoxyethylene-9,5-octylphenylether or Triton X-114, polyoxyethylene-7,5-octylphenylether or Triton X-100, polyethylene glycol monoalkyl ether or Genapol X-080) are commonly used due to their low cloud point temperature. For example, Triton X-114 has cloud point of 23-25 °C. The general procedure in metal ions extraction by CPE is shown in Figure 1.2.

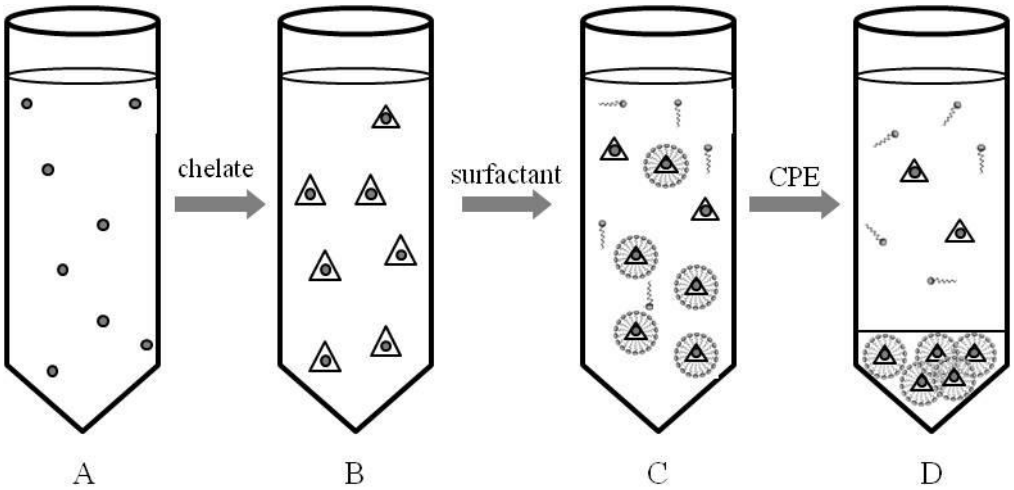


Figure 1.2 Schematic representation of a conventional CPE procedure for metal preconcentration: (A) Sample solution with metal ions (circle); (B) metal chelates (triangles) formed after adding complexing reagent to the sample solution; (C) addition of surfactant in the solution and trapping of metallic chelates into micellar cores; and (D) micellar phase segregates after heating the solution and the phase separation was achieved after centrifugation.

In CPE of metal ions, the target metal ions or metal complexes are partitioned between the surfactant and the aqueous phases. Thus, pertinent partition coefficients of ligands and complexes are essential for quantitative extraction.

2.4 Literature review

CPE has been employed in extraction of silver ions from samples for the determination by spectroscopic techniques such as flame atomic absorption spectrometer (FAAS). The examples are as follows.

Shemirani et al. [26] studied the CPE of Ag(I) ions using 2-mercaptobenzothiazole (MBT) as chelating agent. The extraction was performed by using 0.1% Triton X-114 solution at 60 °C. The silver concentration in surfactant

phase was determined by FAAS. By using 10 mL of water sample, the detection limit was found to be 2.2 μg L^{-1}. The linear range was 10-200 μg L^{-1}.

Tavallali et al. [27] proposed a CPE method for extraction of trace Ag(I) ions by using 1-(2-pyridylazo)-2-naphthol (PAN) as the chelating agent and Triton X-114 as surfactant. After CPE at 55 °C, the surfactant phase was separated and diluted with methanol for the analysis by FAAS using discrete nebulization. The linear range of the method was in a range of 20-500 μg L^{-1}. The method provided an enrichment factor of 43 with the detection limit of 6.5 μg L^{-1}.

Liu et al. [28] proposed a CPE method for separation and preconcentration of AgNPs in environmental water sample by using $Na_2S_2O_3$ to enhance the separation of AgNPs from Ag^+. Charged complex of Ag^+ and $S_2O_3^{2-}$ was not extracted into the surfactant rich phase. The effect of the presence of humic acid, Ag^+ concentration and AgNPs size were also studied. An enrichment factor of 100 was obtained with 0.2% (w/v) TX-114, and the recoveries of AgNPs from various environmental samples were in the range of 57-116% at 0.1-146 μ L^{-1} spiked levels. The method gave detection limit of 0.006 μg L^{-1}.

Chao et al. [11] proposed the method for speciation analysis of silver nanoparticles and silver ions in antibacterial products and environmental water samples. $Na_2S_2O_3$ was used to form hydrophilic complex with Ag^+ and remained in the aqueous phase. AgNPs was extracted into the surfactant phase and determined by ICP-MS after being digested. The amount of total Ag was directly dertermined by ICP-MS. The amount of Ag^+ was obtained by subtracting the amount of total Ag by the amount of AgNPs. The limits of quantification were 0.4 μg kg^{-1} and 0.2 μg kg^{-1} for AgNPs and total Ag, respectively. The spiked recoveries of AgNPs were in the range of 71.7 – 103%.

CHAPTER III

EXPERIMENTAL

3.1 Instruments

The instruments used in this research are listed in Table 3.1. The operating parameters for silver ion determination by flame atomic absorption spectrometer are shown in Table 3.2.

Table 3.1 Instruments list

Instrument name	Model (company)
1. Transmission electron microscopy (TEM)	JEM-2100; JEOL, Japan Fisher
2. Flame atomic absorption spectrometer (FAAS)	AAnalyst 100 (Perkin Elmer)
3. UV-Vis spectrophotometer	HP 8453 (Hewlett Packard)
4. Centrifuge	Universal 320; HETTICH
5. pH meter	Seven compact model; Mettler TOLEDO
6. Magnetic stirrer	MS101; GEM Magnetic stirrer
7. ISE – Silver electrode	In-house made electrode [29]

Table 3.2 Operating condition for determination of silver ion by FAAS

Wavelength	328.30 nm
Slit width	0.70 nm
Lamp current	15.0 mA
C_2H_2 flow-rate	2.0 L min^{-1}
Air flow-rate	4.0 L min^{-1}

3.2 Chemicals

All chemicals used in this research are of analytical grade and used without further purification (Table 3.3).

Table 3.3 Chemicals list

Chemicals	Supplier
Hydrogen peroxide	Merck
Trisodium citrate dihydrate	Fisher chemicals
Ag(I) standard solutions (1000 mg L^{-1})	BDH
Nitric acid (65% w/w)	Merck
Sodium hydroxide	Merck
Sodium thiosulfate	Merck
L-Cysteine hydrochloride	Sigma Aldrich
1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol, Polyethylene glycol *tert*-octylphenyl ether (Triton X-114)	Sigma Aldrich
Ethanol	Merck

3.3 Preparation of solutions

Silver nitrate solutions

Silver nitrate solution (1.0 mM) was prepared by dissolving an appropriate amount of silver nitrate in de-ionized water and used for silver nanoparticles preparation.

Citrate solution

Citrate solution (1% w/v) was prepared by dissolving an appropriate amount of trisodium citrate dihydrate in de-ionized water and used as reducing agent for silver nanoparticles preparation.

Sodium thiosulfate solution

Sodium thiosulfate solution was prepared freshly before use by dissolving sodium thiosulfate in de-ionized water to obtain the solutions with concentration in the range of 5–1000 mM. These solutions were used for investigating the suitable thiosulfate concentration in the separation of silver nanoparticles and silver ions during cloud point extraction.

Silver standard solutions

Silver standard solutions were prepared by dilution of 1,000 mg L^{-1} stock standard solution to desired concentrations with de-ionized water.

Nitric acid solutions

Nitric acid solutions (1% v/v) were prepared by diluting the concentrated nitric acid (65% w/w) with de-ionized water and used for solution pH adjustment.

Sodium hydroxide solutions

Sodium hydroxide solutions (1% w/v) was prepared by dissolving an appropriate amount of sodium hydroxide pallet in de-ionized water and used for solution pH adjustment.

L-cysteine solutions

L-cysteine solution was prepared by dissolving an appropriate amount of L-cysteine in DI water.

Triton X-114 solution

Triton X-114 solution (10% w/v) was prepared by dissolving 10 grams of Triton X-114 in DI water.

3.4 Preparation and characterization of silver nanoparticles

Silver nanoparticles (AgNPs) solutions were prepared by the method of citrate reduction of $AgNO_3$ solution. A portion of 50 mL of $AgNO_3$ solution (1.0 mM) was heated to boiling. Then, 5 mL of 1% trisodium citrate solution was added drop by drop to the solution. The mixture was stirred continuously and vigorously and heated until its color changed to pale yellow. The colloidal solution was removed from heating element and stirred until cooled to room temperature. Then, the obtained solution was centrifuged at 14000 rpm for 20 min, the colloidal AgNPs were separated and redispersed in de-ionized water for further use as stock solution of AgNPs. The AgNPs stock solution were kept in the dark and used within one week. The absorption spectra and the particles size of the obtained AgNPs were measured by UV-Visible spectrophotometer and transmission electron microscopy (TEM), respectively.

3.5 Measurement of Ag^+ and total silver in AgNPs stock solution.

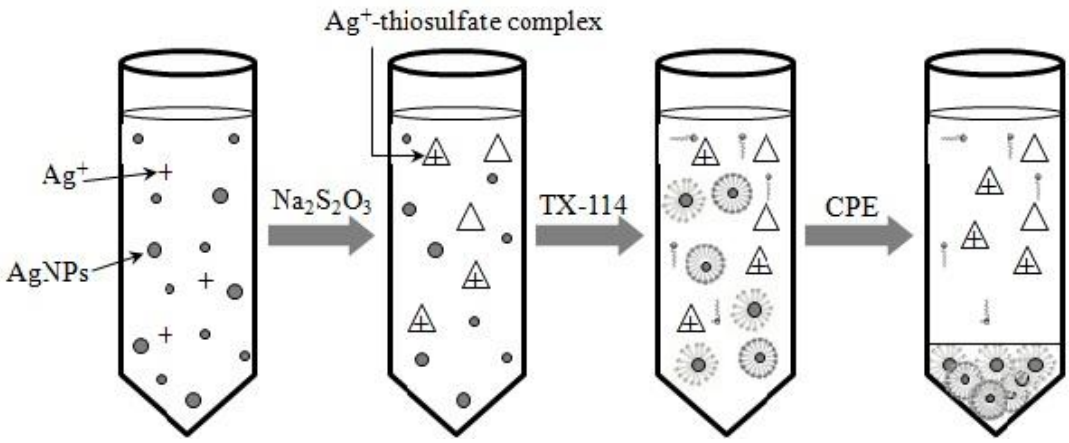
The free Ag+ concentration in the prepared AgNPs stock solution was determined with Ag^+-ion selective electrode. The signal was collected and compared to standard calibration curve. The amount of total Ag in AgNPs solution was determined by adding 0.2 mL of H_2O_2 (30%) into 10 mL of AgNPs stock solution and determined with Ag^+-ion selective electrode. The amount of AgNPs in stock solution was obtained by substraction the amount of total silver by that of silver ions.

3.6 Procedure of cloud point extraction

3.6.1 Procedure of AgNPs determination

A portion of 1.0 mL of 10% (w/v) Triton X-114 solution was added to an aliquot of 25.0 mL of sample solution. The pH of the solution was adjusted to the desired value with HNO_3. The mixture was incubated in a thermostatic bath for 30 min at 40 °C. Then the phase separation was accelerated by centrifugation for 10 min

at 3500 rpm. The two phases were cooled down in an ice bath and the aqueous phase was decanted. 1 mL of concentrated HNO_3 and 1 mL of 30% H_2O_2 were added to the surfactant phase in the tube to digest the nanoparticles. Then, 100 μL of ethanol were added to reduce the viscosity and dissolve the remaining surfactant droplets. The mixture volume was made up to 5 mL with de-ionized water. Then, the silver concentration was determined by FAAS. All experiments were performed in triplicate.



Scheme 3.1 The step of CPE for extraction of AgNPs.

3.6.1.1 Effect of $Na_2S_2O_3$ concentration

In the determination of AgNPs, the effect of $Na_2S_2O_3$ concentration was studied. A 0.1 mL of $Na_2S_2O_3$ of different concentration ranging from 2-1000 mM was added to sample solution containing 1.0 mg L^{-1} AgNPs and 0.06 mg L^{-1} Ag^+ (pH 5). Then, the cloud point extraction and silver concentration determination were performed as described previously.

3.6.1.2 Effect of pH

For determination of AgNPs in sample, the effect of pH was studied. The pH of a 25.0 mL aliquot of sample solution containing 1.0 mg L^{-1} AgNPs and 0.06 mg L^{-1}

Ag^+ was adjusted by 1% HNO_3 and/or 1% NaOH to the values in the range of 2.5 to 6.0. Then, 0.1 mL of 20 mM of $Na_2S_2O_3$ was added to sample solution before the CPE was performed.

3.6.1.3 Effect of AgNPs size

The effect of AgNPs size was studied. The pH of a 10.0 mL aliquot of sample solution containing AgNPs with the size of 18-50 nm in diameter (AgNPs prepared by citrate reduction method) or 4-12 nm in diameter (commercial AgNPs) was adjusted to pH 3 with 1% HNO_3. A solution of 10 mM of $Na_2S_2O_3$ (0.1 mL) was used in this experiment. CPE and silver concentration determination were carried out as described previously.

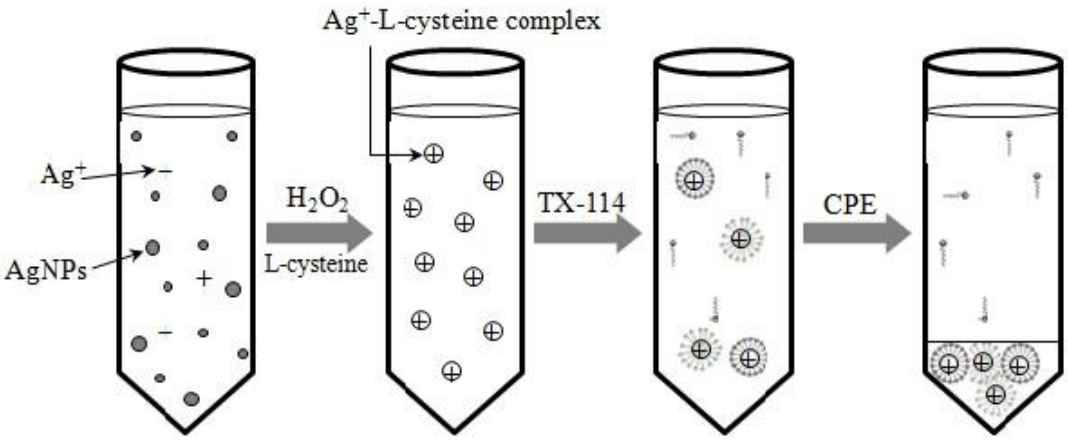
3.6.1.4 Effect of Ag^+ concentration

The effect of Ag^+ concentration was investigated by extraction of AgNPs from solution containing AgNPs and Ag^+ in different mole ratio. A 10.0 mL aliquot of sample solution containing 0.93 mg L^{-1}AgNPs and Ag^+ in concentration of 1.0 mg L^{-1} or 3.0 mg L^{-1} was used. Then, 0.1 mL of 10 mM $Na_2S_2O_3$ solution was added to the sample solution. CPE and silver concentration determination were performed as explained previously.

3.6.2 Procedure of total Ag determination

In the determination of total Ag, 1.0 mL of 30% H_2O_2 was added to 25.0 mL of sample solution to convert AgNPs to Ag^+ ions. Then, 0.1 mL of L-cysteine solution and 1.0 mL of 10% (w/v) Triton X-114 solution were added. The mixture was incubated in a thermostatic bath for 30 min at 40 °C. Then the phase separation was accelerated by centrifugation for 10 min at 3500 rpm. The two phases were cooled down in an ice bath and the aqueous supernatant phase was decanted. A 0.1 mL portion of concentrated HNO_3 was added to the surfactant phase in the tube. Then, 0.1 mL of ethanol were added to reduce the viscosity and dissolve the remaining

surfactant droplets. The mixture volume was made up to 5 mL with de-ionized water. Then, the silver concentration was determined by FAAS. All experiments were performed in triplicate.



Scheme 3.2 The step of CPE for extraction of total Ag^+.

3.6.2.1 Effect of L-cysteine concentration

Solutions of L-cysteine in concentration ranging from 2-100 mM were added to sample solutions. The sample solution contained 0.87 mg L^{-1} total Ag. The solution pH was adjusted to pH 5. Then, CPE and the determination of total silver concentration were performed as described previously.

3.6.2.2 Effect of pH

To obtain the quantitative extraction, solution pH is an important parameter. In the determination of total Ag, the solution pH was adjusted by using 1% HNO_3 and/or 1% NaOH to a value in the range of 1 to 8. Then, 0.1 mL of 20 mM L-cysteine was added to sample solution containing 0.70 mg L^{-1} total Ag. The mixture was extracted and silver concentration was determined as described previously.

3.6.2.3 Effect of co-existing ions

The effect of co-existing ions was investigated by comparing the amount of extracted Ag^{+} in the absence and presence of other metals such as Zn^{2+},Pb^{2+}, Ni^{2+}, Cu^{2+} and Hg^{2+} in the concentration of 5 or 50 mg L^{-1}. Each of these metals ions solutions was added to a 25.0 mL aliquot of sample solution containing 0.5 mg L^{-1} AgNPs to prepare binary mixture. The solution pH was adjusted by 1% HNO_3 and/or 1% NaOH to pH 3. Then, 0.1 mL of 20 mM L-cysteine was added prior to CPE was performed. The silver concentration was determined by FAAS.

3.7 Method validation

Method validation was performed by using sample spiked with stock standard AgNPs solution under two concentration levels: 0.5 and 1.0 mg L^{-1}. The analysis was repeated 9 times at the optimum conditions. The accuracy and precision were represented in term of the percentage of recovery and the relative standard deviation (%RSD), respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the standard deviation of 9 measurements of blank solution by FAAS without CPE.

3.8 Real sample analysis

To test the applicability of the CPE method in the determination of AgNPs and total Ag in real samples, the real samples were spiked with the stock standard solution of AgNPs at two concentration levels: 0.5 and 1.0 mg L^{-1}.

The water samples used in this experiment were pond water, drinking water and tap water, spiked with AgNPs at 0.5 and 1.0 mg L^{-1}. Amount of total Ag and AgNPs was determined by the propose method at optimize condition. The accuracy and precision were reported as %recovery and %RSD.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, a method for separation and determination of silver nanoparticles (AgNPs) and total silver was proposed. The effect of parameters that have influence in the extraction was demonstrated as follows.

4.1 Determination of silver nanoparticles (AgNPs)

4.1.1. Effect of $Na_2S_2O_3$ concentration

In this method, $Na_2S_2O_3$ was used as masking agent to form charged complexes with Ag^+ ions and enhance the separation of AgNPs from Ag^+ during cloud point extraction. Figure 4.1 shows the recovery of silver in surfactant phase observed and compared to starting amount of AgNPs when various concentrations of $Na_2S_2O_3$ were added into sample solution. In the absence of $Na_2S_2O_3$, some of Ag^+ were likely to absorb on AgNPs surface and extracted into surfactant phase, resulting in a high recovery value. On the other hand, the recovery of silver in surfactant phase decreased in the presence of $Na_2S_2O_3$ and reached relatively constant values when $Na_2S_2O_3$ concentration was 10 mM and higher. These results can be explained by the formation of hydrophilic charged complex between Ag^+ and $S_2O_3^{2-}$, which remained in aqueous phase during extraction. $Na_2S_2O_3$ concentration of 20 mM was chosen for the separation of Ag^+ and AgNPs. However, the observed recovery of AgNPs from solution at this pH was still low and the effect of pH was further investigated.

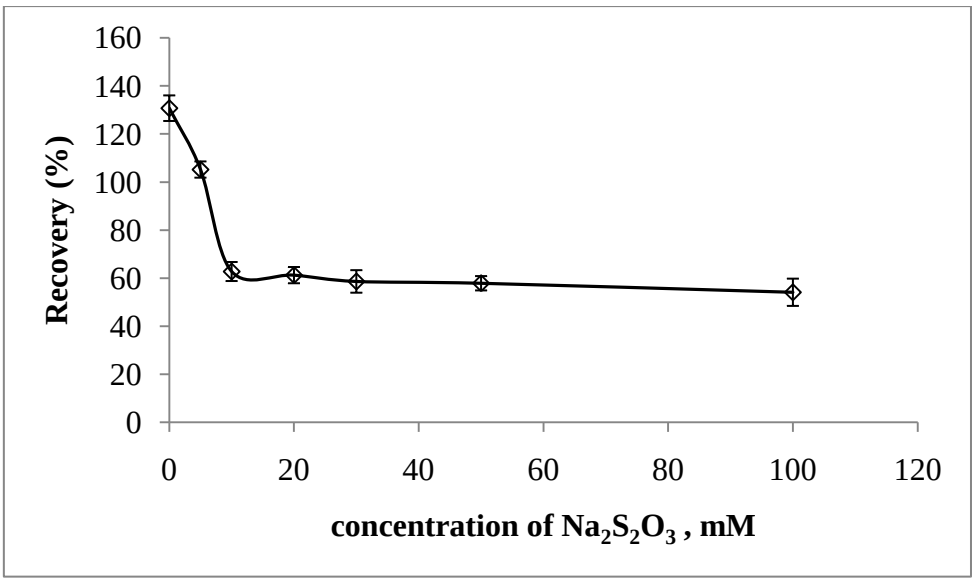


Figure 4.1 Recovery of silver in surfactant phase after cloud point extraction using various concentrations of $Na_2S_2O_3$ in sample (0.8 mg L^{-1} AgNPs and 0.06 mg L^{-1} Ag^+) at pH 5.

4.1.2. Effect of pH

The effect of sample pH on silver nanoparticles extraction was evaluated in a range of pH 2.5 to 6.0. A solution of 20 mM $Na_2S_2O_3$ was added into sample solution. The recovery of silver in surfactant phase increased with an increase of sample acidity (Figure 4.2). It is likely that in acid solution, the surface of AgNPs where citrate ions were adsorbed became more hydrophobic due to the protonation of citrate capping agent (pK_a values of citric acid, pK_{a1} = 3.13, pK_{a2} = 4.76, pK_{a3} = 6.40) [30]. As a result, AgNPs could be extracted into surfactant phase in higher amount when the solution pH was decreased from 6.0 to 2.5. However, the extraction at pH < 3.0 is not applicable in this proposed method due to the possible dissolution of AgNPs [28], as well as the decomposition of $S_2O_3^{2-}$ and the formation of sulfur precipitation [31]. The suitable pH for AgNPs extraction was pH 3, hence the sample solution pH should be adjusted to pH 3 prior to cloud point extraction.

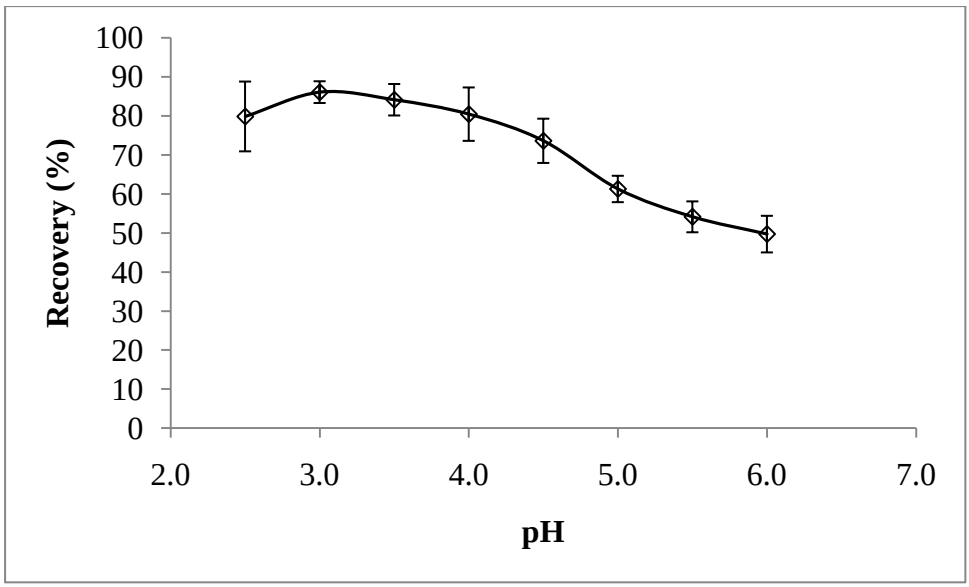


Figure 4.2 Recovery of silver in surfactant phase after cloud point extraction of AgNPs from solution having different pH (1.0 mg L^{-1} AgNPs and 0.06 mg L^{-1} Ag^{+}, 20 mM $Na_2S_2O_3$).

The extraction of AgNPs into surfactant phase was also confirmed by TEM images (Figure 4.3).

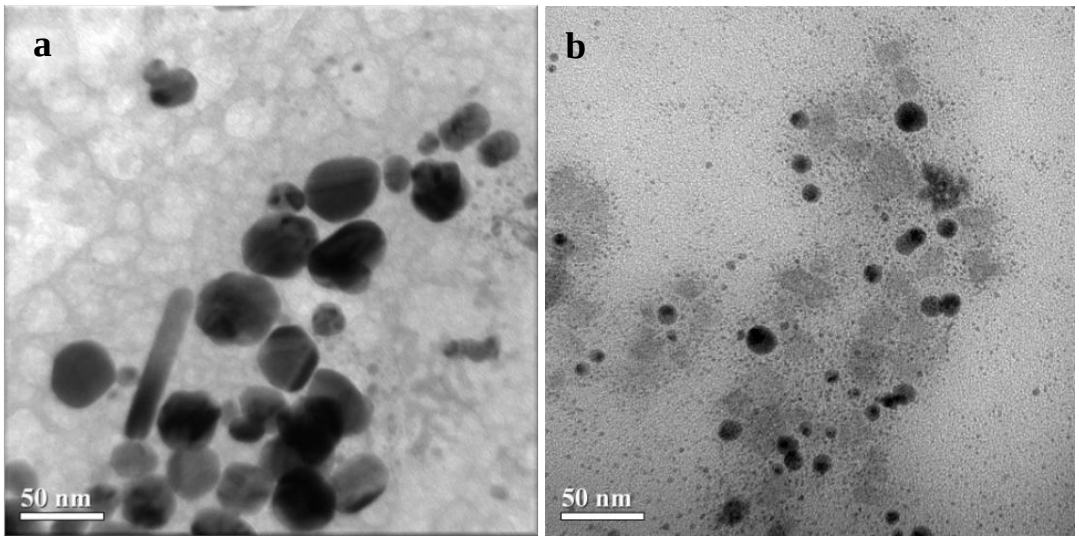


Figure 4.3 TEM images of AgNPs in (a) aqueous solution before extraction and (b) surfactant rich phase after extraction.

4.1.3. Effect of AgNPs size

The AgNPs sample with different particle size and capping reagents were tested under the chosen conditions (pH 3). Samples of AgNPs used in this study was prepared by citrate reduction method and compared to commercial AgNPs with unknown capping reagent. The UV spectra are shown in the Figure 4.4. The particle size of AgNPs was determined by TEM. It is clearly seen that commercial AgNPs solution contained AgNPs with the particle size much smaller than that of AgNPs obtained from citrate reduction method.

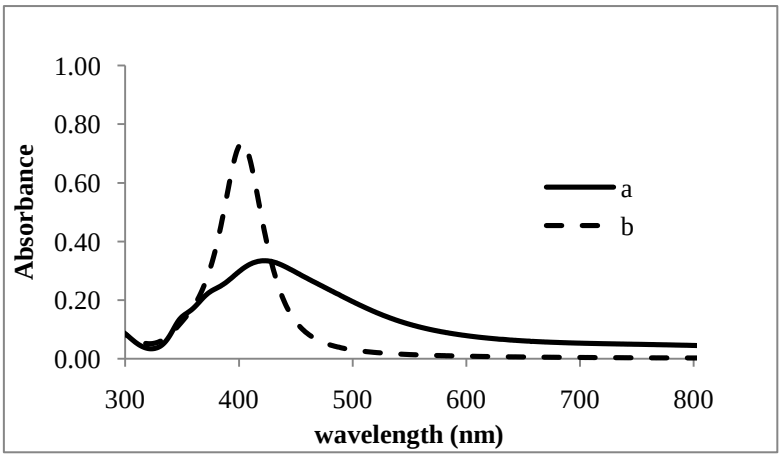


Figure 4.4 UV-Vis absorption spectra of (a) silver nanoparticles obtained from citrate reduction and (b) commercial silver nanoparticles (AgNPs-commercial).

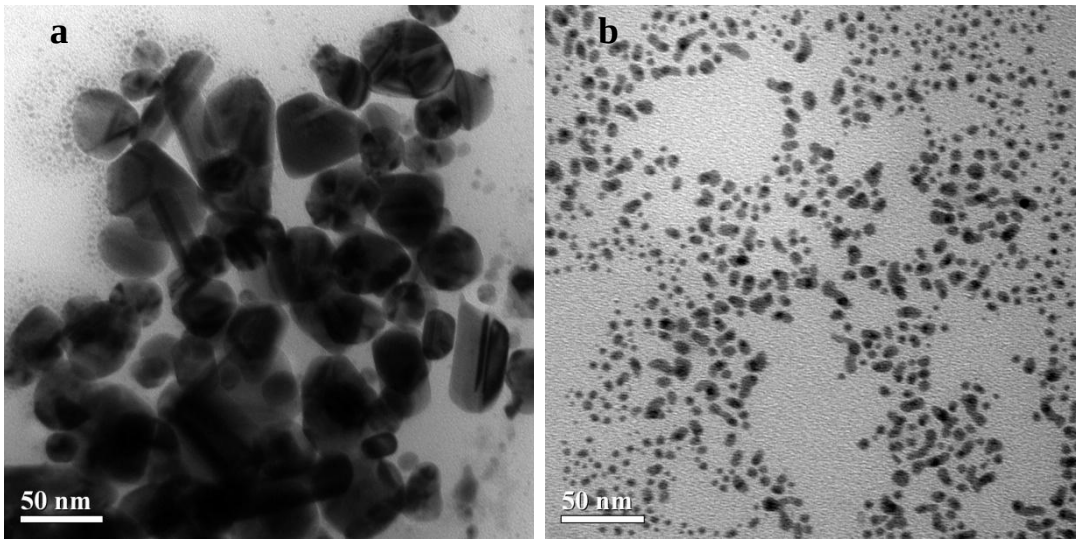


Figure 4.5. TEM images of (a) AgNPs from citrate reduction (b) commercial AgNPs.

The results of cloud point extraction are shown in Table 4.1. Under chosen condition, only 44.27 % of AgNPs in commercial solution were recovered. The commercial AgNPs used in this study contained unknown stabilizer, and therefore it is likely that the pH of solution used in the extraction was not suitable for quantitative extraction of commercial AgNPs. It is probable that the low recovery of commercial AgNPs at chosen condition might be due to the dissolution of small size AgNPs. Then, the released Ag^+ ions were masked by $S_2O_3^{2-}$ and remained in the aqueous phase. Zhang et al. [32] also proposed that smaller size of AgNPs dissolve more rapidly than large particles. The larger surface area of smaller particles, the more surface atoms are exposed and available for oxidation, resulting in subsequent dissolution. Furthermore, not only the particle size but also the coating type and synthesis method are related to the dissolution of AgNPs [33]. In the previous researches, different condition for quantitative extraction of AgNPs was reported. Liu et al. [28] proposed the extraction of AgNPs (9-73 nm i.d.) prepared by using sodium hypophosphite and PVP as reducing agent and stabilizer from solution at pH 3. On the other hand, Hartmann et al. [37] observed high extraction efficiency of AgNPs prepared by using $NaBH_4$ from solution at pH ≥ 5 using acetate buffer. These different observations were probably due to the different surface modifications of the particles, leading to different in zeta potential of the particles and pH at point of zero charge (pH_{pzc}) [28].

Table 4.1 Recovery of AgNPs of different size from solution

Samples	Estimated particle size (nm)	AgNPs concentration (mg L^{-1})	Recovery (%)
AgNPs (citrate reduction)	18-50	0.89	86.09 ± 2.09
Commercial AgNPs	4-12	0.62	44.27 ± 5.39

4.1.4. Effect of Ag^+ concentration

In the proposed method, Ag^+ ions in sample solution would form negatively charged metal-ligand complexes with $Na_2S_2O_3$ and remain in aqueous phase. Under the chosen condition, an increasing of mole number of Ag^+ over the mole of AgNPs may enhance the adsorption of the coexisting Ag^+ ions on the surface of AgNPs and hence the extraction of Ag^+ together with AgNPs. This could result in positive errors. Therefore, the effect of Ag^+ concentration was studied by using samples containing Ag^+ less than AgNPs amount and 3 times higher than AgNPs amount. The recovery of AgNPs when the concentration of Ag^+ was not over the concentration of AgNPs (AgNPs 0.93 mg.L^{-1}, Ag^+ 1.0 mg L^{-1}) was 81.09 ± 7.78 %. On the other hand, the recovery of AgNPs was 189.87 ± 31.59 % when the concentration of Ag^+ in solution was over 3 times of AgNPs (AgNPs 0.93 mg L^{-1}, Ag^+ 3.0 mg L^{-1}). These results were also observed by Liu et al. [28].

4.2 Determination of total Ag

To determine total silver concentration, the sample solution was digested with H_2O_2 to oxidize AgNPs to Ag^+ ions. L-cysteine was added into sample solution to enhance the extraction of silver as silver-cysteine complexes into surfactant phase prior to the determination by FAAS [35-37].

4.2.1. Effect of L-cysteine concentration

The concentration of L-cysteine over a range of 2 to 100 mM was evaluated. The results are shown in Figure 4.6. The recovery of silver in surfactant rich phase increased after adding L-cysteine of concentration of 2 mM and 20 mM and decreased after 50 mM L-cysteine was added into the solution. It could be explained by the formation of charged complex of L-cysteine and Ag^+ in solution when high concentration of L-cysteine was used Therefore, charged silver complexes remained in aqueous phase during extraction. In this research, the concentration of L-cysteine of 20 mM was chosen for the extraction of Ag^+ from solution.

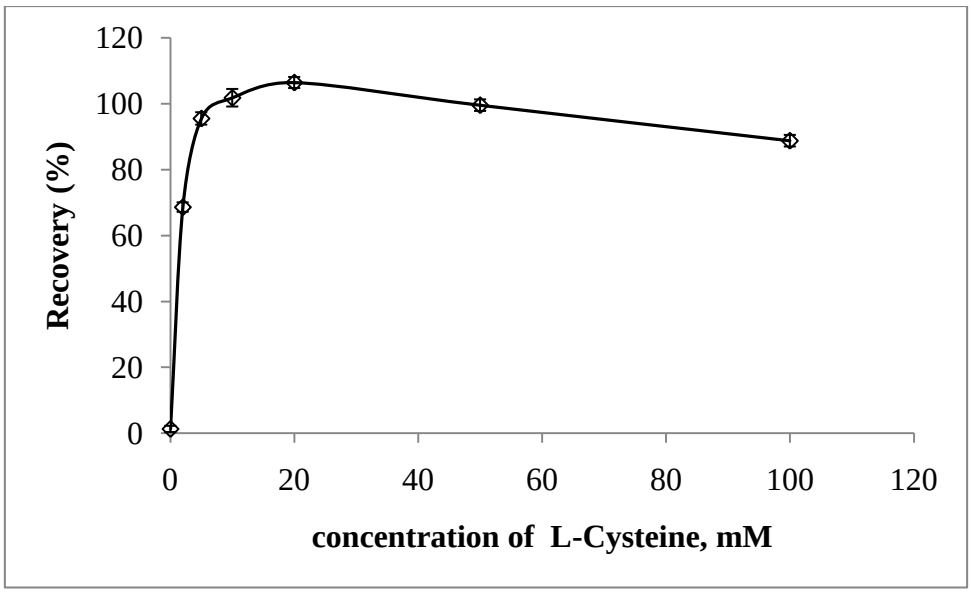


Figure 4.6 Recovery of silver in surfactant phase after cloud point extraction using various concentration of L-cysteine in sample (0.8 mg L^{-1} total Ag from solution) at pH 5.

4.2.2. Effect of sample pH

Solution pH plays an important role in metal complex formation. In this research, the extractions were performed using solution with pH value ranging from 1 to 8. Metal ions could be separated and extracted into a surfactant rich phase by formation of hydrophobic complex with 20 mM L-cysteine. Figure 4.7 shows the effect of pH on the extraction of Ag^{+}.

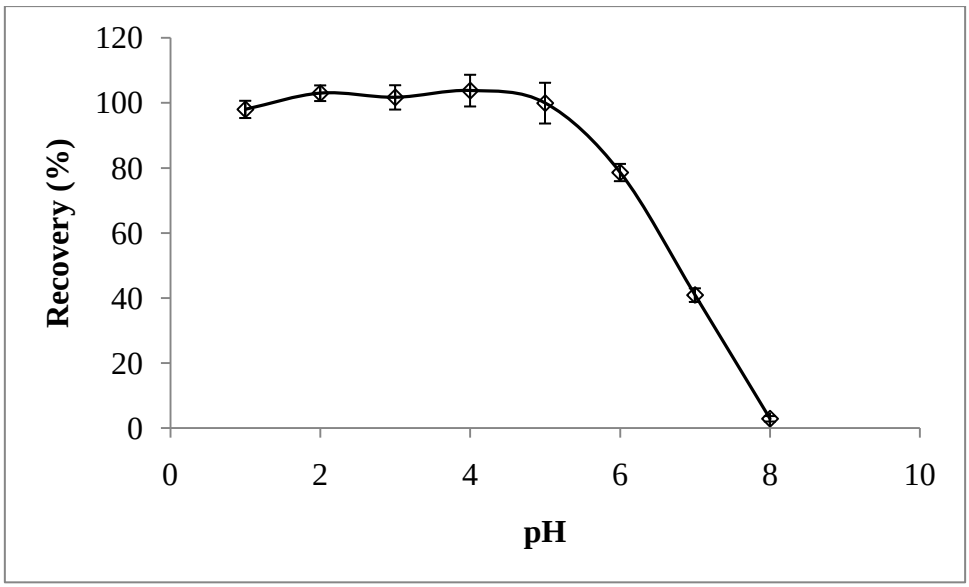


Figure 4.7 Recovery of silver in surfactant phase after cloud point extraction of Ag^+ ions from solution having different pH (0.7 mg L^{-1} total Ag, 20 mM L-cysteine).

It was found that in a pH range of 1 to 5, the recovery of total silver ions was achieved. The decrease of silver ions recovery from solution with pH ≥ 6 was probably due to the formation of charged complex of L-cysteine and Ag^+ in solution. The possible complexation equilibria that may occur were proposed by Alekseev et al. [38] and shown in the following equations. The proposed structures are shown in Figure 4.8.

$$Ag^+ + Cys^{2-} \leftrightarrows AgCys^-$$
$$Ag^+ + H^+ + Cys^{2-} \leftrightarrows AgHCys$$

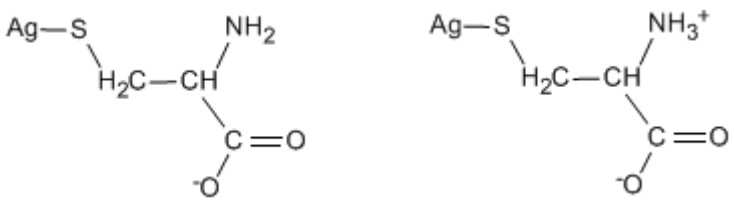


Figure 4.8 Structure of the $AgCys^-$(left) and AgHCys (right) complexes [38].

Considering the pK_a values of L-cysteine (pK_a of carboxylic acid = 2.00, pK_a of alpha amino = 8.11 and pK_a of side chain = 10.10, [39]), in solution of pH 1-5, silver ions may form neutral complex with L-cysteine (AgHCys) that could be extracted into surfactant phase. When the solution pH was higher 5, the alpha amino groups would be less protonated and the silver-cysteine complexes may be in form of charged complexes ($AgCys^-$). As a result, the charged silver complexes were not extracted into surfactant phase.

4.2.3. Effect of co-existing ions

To study the effect of co-existing ions on total silver determination, an individual metal ions solution (5 mg L^{-1} or 50 mg L^{-1}) of Zn(II), Pb(II), Cu(II), Ni(II) or Hg(II) was added into sample solution containing 0.5 mg L^{-1} total Ag^+. The recoveries of Ag^+ ions from samples (Table 4.2) indicate that the presence of these co-existing ions except Cu^{2+} in a concentration of 5 mg L^{-1} did not affect the extraction of Ag^+, compared to those observed in the absence of co-existing ions. In solution containing 50 mg L^{-1} of co-existing ion, only the presence of Cu^{2+} and Hg^{2+} affected strongly the recovery of Ag^+ ions. It can be explained that L-cysteine could also form complex with these metal ions [38]. The stability constants are shown in Table 4.3. When the concentration of these metal ions was increased, the competition between Ag^+ and other metal ions in complex formation with L-cysteine occurred and L-cysteine tended to form complexes with metal ions (i.e. Cu^{2+} [40], Hg^{2+}) other than Ag^+, resulting in low recovery of Ag ions in surfactant phase. Hg^{2+} ions form complexes with L-cysteine with the highest stability constant, compared to other studied metal ions, and hence show strong effect on Ag^+ ions extraction.

Table 4.2 Effect of co-existing ions on the determination of Ag(I) by FAAS

Co-existing ion	Concentration (mg L^{-1})	Recovery (%)
Without co-existing ion	-	89.79 ± 2.18
Zn^{2+}	5	86.77 ± 1.60
	50	85.77 ±5.96
Pb^{2+}	5	90.39 ±2.41
	50	81.34 ±1.81
Cu^{2+}	5	4.05 ± 1.04
	50	2.03 ± 0.35
Ni^{2+}	5	90.19 ± 2.12
	50	80.94 ± 6.65
Hg^{2+}	5	87.57 ± 0.92
	50	33.63 ±14.83

Table 4.3 Formation constant for L-cysteine metal complex [41]

Metal ion	Complex *	logβ
Ag^{+}	ML	14.0
Zn^{2+}	ML_2	18.2
	ML	9.86
Pb^{2+}	ML	12.20
Cu^{2+}	ML_2	16.0
Ni^{2+}	ML_2	19.3
	ML	10.48
Hg^{2+}	ML	20.5
	ML_2	43.57
	ML_2H	54.37
	ML_2H_2	61.79

* M = metal ions, L = L-cysteine ligand

4.3 Method validation

The chosen method was validated by demonstration the performance of the method related to accuracy and precision which are reported in term of %recovery and relative standard deviation of the results obtained (%RSD), respectively. The accuracy was examined by analyzing sample with known concentration of AgNPs and Ag^+ and the percentage of recovery values were calculated by using Equation (4.1);

$$\%\,Recovery = \frac{N_{bs}-N_b}{N_s}\,x\,100 \qquad (4.1)$$

where N_{bs} = The amount of metal ion determined in a spiked sample (mg)

N_s = The amount of metal ion that was spiked (mg)

N_b = The amount of metal determined in blank or unspiked sample (mg)

The precision of this method was examined in term of percent relative standard deviation (%RSD) under chosen conditions, which is calculated by equation (4.2);

$$\%\,RSD = \frac{SD}{\overline{X}}x100 \qquad (4.2)$$

where SD = standard deviation of results

$\overline{X}$ = mean value of results

The standard calibration curves used in the determination of AgNPs were constructed by analyzing standard solutions containing 1 mL of 30% H_2O_2, 0.1 mL of EtOH and 1 mL of conc. HNO_3, while the standard calibration curves for determining total Ag^+ were obtained by analyzing standard solutions containing 0.1 mL EtOH and 0.5 mL of conc.HNO_3. Different reagents were used in order to either dissolve AgNPs or Ag-cysteine complex.

The method detection limit was determined by using the results from blank solution analysis. The limit of detection (LOD) is the lowest concentration level of an analyte in the sample that show the signal equal to mean value of blank solution

signal (y_b) plus three times of blank signal standard deviation (SD) as shown in Equation (4.3). The signal of LOD was compared to external calibration curve to obtain LOD concentration.

$$S_{LOD} = y_b + 3SD \quad (4.3)$$

Accuracy and precision in standard solution and sample analysis are summarized in Table 4.4. The amount of AgNPs and total Ag in water samples and spiked samples were determined by the proposed method. The real samples for this work were water in a pond, drinking water and tap water.

Table 4.4 Determination of total Ag and AgNPs in standard solutions and real sample by the proposed method (mean ± SD), n=3

Sample	Added [a] (mg L^{-1})		Found (mg L^{-1})		Recovery (%)		RSD
	Total Ag	AgNPs	Total Ag	AgNPs	Total Ag	AgNPs	(%)
Standard	-	-	BLR	BLR	-	-	-
	0.19	0.50	NA	0.45±0.01	-	87.95	3.06
	0.38	1.02	NA	0.88±0.03	-	86.74	3.38
	0.50	-	0.49±0.01	NA	95.76	-	2.40
	1.00	-	0.94±0.03	NA	93.31	-	3.15

Table 4.4 Determination of total Ag and AgNPs in standard solutions and real sample by the propose method (mean ± SD), n=3 (continued)

Sample	Added [a] (mg L^{-1})		Found (mg L^{-1})		Recovery (%)		RSD
	Total Ag	AgNPs	Total Ag	AgNPs	Total Ag	AgNPs	(%)
Pond 1	-	-	BLR	BLR	-	-	-
	0.19	0.50	NA	0.41±0.03	-	81.25	7.02
	0.38	1.02	NA	0.84±0.06	-	82.55	6.82
	0.50	-	0.43±0.02	NA	83.59	-	4.87
	1.00	-	0.82±0.04	NA	81.95	-	5.14
Pond 2	-	-	BLR	BLR	-	-	-
	0.19	0.50	NA	0.40±0.04	-	77.89	10.76
	0.38	1.02	NA	0.74±0.09	-	73.32	11.72
	0.50	-	0.65±0.04	NA	58.54	-	12.31
	1.00	-	0.65±0.07	NA	65.11	-	10.98
Tap water	-	-	BLR	BLR	-	-	-
	0.19	0.50	-	0.42±0.02	-	82.08	5.11
	0.38	1.02	-	0.83±0.03	-	82.12	5.14
	0.50	-	0.44±0.02	NA	85.02	-	3.80
	1.00	-	0.82±0.04	NA	81.95	-	3.82
Drinking water	-	-	BLR	BLR	-	-	-
	0.19	0.50	-	0.44±0.01	-	86.28	3.41
	0.38	1.02	-	0.86±0.03	-	84.22	3.74
	0.50	-	0.46±0.01	NA	89.32	-	3.24
	1.00	-	1.02±0.03	NA	101.66	-	2.91

[a] determined by Ag-ISE [29]

BLR = below linear range

NA = not analyzed

It was found that the percentages of recovery of AgNPs and total Ag^+ from sample and %RSD of the results obtained by this proposed method were acceptable at both concentration levels in comparison with acceptable values shown in Table 4.5, except sample from pond 2. This might be due to the presence of organic matrix in real sample that would adsorb AgNPs and Ag^+ or form complexes with Ag^+ and prevent the extraction into the surfactant phase. These results suggest that the sample may need sample preparation (e.g. by acid digestion) before extraction. Method performance is shown in Table 4.6.

Table 4.5 Acceptable values of recovery and precision in the determination at different concentrations [42]

Unit	Mean recovery, %	RSD, %
100%	98-102	1.3
10%	98-102	2.8
1%	97-103	2.7
0.1%	95-105	3.7
100 ppm	90-107	5.3
10 ppm	80-110	7.3
1 ppm	80-110	11
100 ppb	80-110	15
10 ppb	65-115	21
1ppb	40-120	30

Table 4.6 Method performance

Performance	Value (After extracted)
Linear range (mg L^{-1})	0.5-5.0
LOD of AgNPs determination (mg L^{-1})	0.27
LOD of total amount of Ag determination (mg L^{-1})	0.11

CHAPTER V

CONCLUSION

5.1 Conclusion

A method for separation and determination of silver nanoparticles (AgNPs) and total amount of silver by using cloud point extraction (CPE) and flame atomic absorption spectrometry was proposed.

In the determination of AgNPs, sodium thiosulfate ($Na_2S_2O_3$) was used as masking agent to form negatively charged complex with Ag^+ and prevent Ag^+ to be extracted into surfactant rich phase. The concentration of $Na_2S_2O_3$ of at least 20 mM are adequate to separate AgNPs from Ag^+ during CPE of solution containing AgNPs with citrate capping agent and Ag^+ of concentration not over 2 fold of that of AgNPs at pH 3. The success of AgNPs extraction into surfactant rich phase was confirmed by TEM. The amount of AgNPs was determined by FAAS after digestion with H_2O_2. At chosen condition, recovery of AgNPs in a range of 86.74-87.95 % was achieved. The limit of detection (LOD) was 0.27 mg L^{-1}.

In the determination of total amount of silver, the sample was oxidized by H_2O_2 prior to the extraction. L-cysteine was used as complexing agent to form hydrophobic complex with Ag^+ ions. The parameters that affect the recovery are the concentration of L-cysteine, pH of sample solution and co-existing ion. It was found that the concentration of L-cysteine of 20 mM gave a good recovery of silver ions from solution containing 1.0 mg L^{-1} Ag when pH of sample solution was in a range of 1-5. The limit of detection (LOD) was 0.11 mg L^{-1}. The results from the study of co-existing ions effect indicated that Hg^{2+} and Cu^{2+} ions were the potential interfering ions in Ag^+ determination. The use of masking agents is recommended.

5.2 Suggestion of future work

- The method sensitivity should be improved in order to be able to determine trace silver ions and AgNPs in sub μg L^{-1} level.
- The effect of co-existing ion should be minimized to improve the accuracy of the method in total amount of Ag determination.

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