

A Sensitive Colorimetric Detection of Ascorbic Acid in Pharmaceutical Products Based on Formation of Anisotropic Silver Nanoparticles

M.R. Hormozi Nezhad^{1,2,*}, M.A. Karimi³ and F. Shahheydari^{3,4}

Abstract. A sensitive colorimetric method for the detection of ascorbic acid was proposed in this research based on the reduction of silver ions by ascorbic acid in the presence of citrate-stabilized silver seeds, additional trisodium citrate and a polymer such as polyvinylpyrrolidone. The color of the stable sol is controlled by varying the concentration of trisodium citrate (TSC), polyvinylpyrrolidone, silver nitrate and silver seeds. The reduction of Ag^+ to triangle silver nanoparticles (Ag-NPs) by ascorbic acid in the presence of trisodium citrate (TSC) and silver seeds produced two very intense surface plasmon resonance peaks of Ag-NPs. The plasmon absorbance of Ag-NPs allows the quantitative spectrophotometric detection of the ascorbic acid. The calibration curve derived from the changes in absorbance at $\lambda = 427$ nm was linear, with the concentration of ascorbic acid in the range of 6.0×10^{-6} to 8.0×10^{-5} M. The method was applied to the determination of ascorbic acid in pharmaceutical formulations with satisfactory results.

Keywords: Triangular silver nanoparticles; Surface plasmon band; Ascorbic acid.

INTRODUCTION

Ascorbic acid is present in both animal and plant kingdoms [1]. Among animal organs, the liver, leukocytes and anterior pituitary lobe have the highest concentration of ascorbic acid [1]. Ascorbic acid is a vital vitamin in the diet of humans and has been used for the prevention and treatment of the common cold, mental illness, infertility, cancer (because it has been identified as a radical scavenger in vivo) and AIDS [2]. Also, the deficiency of this vitamin leads to many diseases like Scurvy (Scurvy is a disease resulting from a deficiency of vitamin C) [3]. Therefore, the analysis of food products and pharmaceuticals containing this vitamin assumes significance, and it is essential to develop a simple and rapid method for its determination in

4. Education Organization in Kerman, Kerman, Iran.

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routine analysis. Though the literature is sated with different types of method for the analysis of various products, efforts continue in the search for even better methods.

Noble metal nanoparticles made of silver and gold have been the focus of research for many decades as a result of their interesting optical properties [4-6]. When silver and gold nanoparticles are dispersed in liquid media, these nanoparticles exhibit a strong UVvis extinction band that is not present in the spectrum of the bulk metal. This extinction band results when the incident photon frequency is resonant with the collective excitation of the conduction electrons, and is known as the Surface Plasmon Resonance (SPR) [6,7]. The plasmon resonance absorption of silver and gold nanoparticles has molar extinction coefficients ($\sim 3 \times 10^{11} \text{ M}^{-1} \text{ cm}^{-1}$) [8], which allow higher sensitivity in optical detection methods than conventional reagents.

Recently, gold and silver nanoparticles used as a colorimetric detection probe can provide an important method of detection, allowing the detection of analytes to be carried out by the naked eye [9]. Due to the excellent plasmon absorption of noble metal nanoparticles (NPs), especially of gold and silver, recently colorimetric nanoprobes have been developed for

^{1.} Department of Chemistry, Sharif University of Technology, Tehran, P.O. Box 11155-9516, Iran.

^{2.} Institute for Nanoscience and Nanotechnology (INST), Sharif University of Technology, Tehran, Iran.

^{3.} Department of Chemistry & Nanoscience and Nanotechnology Research Laboratory (NNRL), Payame Noor University (PNU), Sirjan, Iran.

 $^{*. \} Corresponding \ author. \ E\text{-mail: } hormozi@sharif.edu$

sensitive and selective detection of metal ions [10,11], proteins [12], glucose [13], neurotransmitters [14], phenolic compounds [15] thiol-containing amino acids [16] DNA [17] and pesticides [18].

Obviously, we can precisely control the wavelengths at which gold and silver nanoparticles absorb and scatter light by controlling their shapes, dimensions and structures (e.g. solid versus hollow) [19]. In addition, it is possible to tailor the magnitude of absorption and scattering coefficients by engineering their geometric parameters (e.g. shape, aspect ratios etc.) [20]. Since anisotropic shapes have greater plasmon resonance extinction coefficients and multiple plasmon bands compared to nanospheres, it is of great importance to develop a method that can use anisotropic noble metal nanoparticles for chemical sensing [21].

In the present study, we use a rapid and straightforward method for colorimetric detection of ascorbic acid, as a mild reducing agent for the generation of triangular silver nanoparticles, in the presence of citratestabilized silver seeds and polyvinylpyrrolidone [22].

The plasmon bands of the generated silver nanoparticles enable the quantitative analysis of the ascorbic acid in different pharmaceutical products.

EXPERIMENTAL

Reagents and Apparatus

All chemicals were from commercial sources (Aldrich, Sigma and Merck) and were used without further purification. Doubly distilled water was used throughout the experiments. Silver nitrate (AgNO₃), sodium borohydride (NaBH₄), ascorbic acid, polyvinylpyrrolidone (PVP, Mw = 40000) and trisodium citrate (TSC) were supplied by Merck. All solutions were used within 1 h after preparation, and the experiments were performed at ambient temperature ($25 \pm 2^{\circ}$ C).

The UV-Vis absorbance spectra were recorded on a PerkinElmer (Lambda 25) spectrophotometer with the use of a 1.0 cm glass cell. The size of the Ag-NPs were characterized by Transmission Electron Microscopy (TEM) using a Zeiss transmission electron microscope operated at an accelerating voltage of 80 kV. The TEM sample was collected by sampling two drops of the solution and casting it onto a carboncoated Cu-grid.

Preparation of the Silver Seeds

A 20 mL volume of aqueous solution containing AgNO₃ $(2.9 \times 10^{-4} \text{ M})$ and TSC $(2.5 \times 10^{-4} \text{ M})$ was prepared and cooled in an ice-bath. To this aqueous solution, NaBH₄ (0.1 M, 0.6 mL) was added drop-wise with vigorous stirring. The solution became bright yellow

immediately. The seeds were then stored in the dark and aged for 2 hours prior to use [22].

Procedure for Determination of Ascorbic Acid

In 25 ml volumetric flasks, 10 ml of 0.5% PVP, 100 μ L of silver seeds, 150 μ L of TSC (2.5 × 10⁻² M), and different concentrations of the ascorbic acid were combined. To this solution, AgNO₃ (0.01 M, 5 × 50 μ L) was added slowly with vigorous stirring. Then, a portion of that solution was transferred within 2 min into a 1 cm spectrophotometric cell to record the absorbance.

Sample Preparation for Real Samples Analysis

At least 10 tablets (vitamin C and multivitamins from Iran Daru Pakhsh) were weighed, ground to a fine powder and mixed. A sample equivalent to approximately 200 mg of ascorbic acid was weighed accurately, transferred into a 250 mL calibrated flask and diluted to volume with water. The mixture was sonicated for 10 min to aid dissolution and then filtered. An appropriate volume of the filtrate was diluted further with water, so that the concentration of ascorbic acid in the final solution was within the working range.

RESULTS AND DISCUSSION

The system in this study is a modified method of that previously reported by Kelly et al. [22] for the rapid production of silver nanoparticles of tunable colors, using a polymeric stabilizer, such as polyvinylpyrrolidone (PVP) and trisodium citrate (TSC). In this approach, we used relatively monodisperse seed particles which were prepared by reduction of silver ions with NaBH₄. In the second step of this process, a milder reducing agent like ascorbic acid is used for reduction of silver ions on silver seeds because a slower growth process is required. The ascorbic acid and silver ions diffuse to the surface of the seed particle where electron transfer takes place, resulting in the formation of a silver atom [22]. Upon addition of ascorbic acid, which acts as reducing agent, silver ions are reduced to silver nanoparticles and then the absorbance characteristic to the plasmon of the Ag-NPs is observed.

Figure 1 shows the absorption spectra of the Ag nanoparticles plasmon that is produced by the ascorbic acid against the reagent blank. The UV-vis spectrum illustrates three peaks located at approximately 760 nm, 410 nm and 330 nm. These values may correspond to the in-plane dipole resonance, the out-ofplane dipole resonance and the out-of-plane quadrupole for triangular nanoplates [23,24], respectively. TEM analysis of the colloidal solution reveals that both triangular and spherical particle morphologies are present



Figure 1. Absorbance spectra of Ag-NPs formed by ascorbic acid under optimum conditions.



Figure 2. TEM images of colloidal nanoparticles prepared under optimum conditions.

(see Figure 2). Based on this peak appearance, a method was proposed for the detection of ascorbic acid. A series of experiments were conducted to establish the optimum analytical conditions for its detection. In this research, criteria for the optimum condition were:

- i) Colloid stability.
- ii) Maximum absorption intensity at plasmon wavelength.
- iii) Reproducibility in colloid formation.

The effects of PVP, TSC and silver ion concentrations and seed volume were investigated to find optimum conditions.

Effect of AgNO₃ Concentration

The effect of $AgNO_3$ concentration on the plasmon absorbance intensity of the Ag-NPs and colloid stability was investigated. As seen from Figure 3, the



Figure 3. Effect of silver ion concentration on the plasmon absorbances of the Ag-NPs; AgNO₃ (2.0×10^{-5} to 1.2×10^{-4} M), PVP (2.0 g/L), Seed (100μ L), TSC (1.5×10^{-4} M) and ascorbic acid (2.0×10^{-4} M).

absorbance intensity was found to increase with increasing the concentration of AgNO₃ in the range of 2×10^{-5} to 1.2×10^{-4} M. A further increase in AgNO₃ concentration caused a decrease in colloidal stability and the rapid formation of bulk silver precipitate. Thus, a concentration of 1×10^{-4} M was selected as the optimum silver nitrate concentration for further studies.

Effect of Trisodium Citrate (TSC) Concentration

Ledwith et al. [22] reported that the color of silver nanoparticles produced by this method can be tuned simply by using different concentrations of TSC. Figure 4 shows the changes of the Uv-visible spectrum and the color of the colloidal silver nanoparticles by different concentrations of TSC over the range of $5.0 \times 10^{-6} - 3.0 \times 10^{-4}$ M. The results showed that plasmon intensity and colloid stability were obtained by using 1.5×10^{-4} M of TSC. Thus, a 1.5×10^{-4} M of TSC was selected for subsequent investigations.

Effect of Seed Volume

Seed volume must be carefully controlled to obtain stable green colloidal silver.

Figure 5 shows the influence of seed volume on the Uv-visible spectrum of the colloidal silver nanoparticles in the range of 50-150 μ L seed. The plasmon peak intensity was found to increase with increasing the volume of seed up to 100 μ L. By further increasing seed volume, aggregation begins as the yellow sol first turns a darker yellow, then violet and eventually grayish, after which the colloid breaks down and particles settle out. Therefore, 100 μ L of silver seed was selected as an optimum volume for determination of ascorbic acid.



Figure 4. Effect of TSC concentration on the plasmon absorbances and color of the colloidal silver nanoparticles; AgNO₃ (1.0×10^{-4} M), TSC ($5.0 \times 10^{-6} - 3.0 \times 10^{-4}$ M), PVP (2.0 g/L), seed ($100 \ \mu$ L), and ascorbic acid (2.0×10^{-4} M).



Figure 5. Effect of seed volume on the absorption spectrum of colloidal silver nanoparticles; AgNO₃ (1.0×10^{-4} M), TSC (1.5×10^{-4} M), PVP (2.0 g/L) and seed (50-150 μ L).

Effect of PVP Concentration

The effects of PVP concentration on the plasmon absorbance intensity and colloidal stability of the silver nanoparticles were studied. It was observed that the addition of PVP within the interval of 1-3 g/L of PVP had no significant effect on the plasmon absorbance and colloidal stability of the silver nanoparticles. A concentration of 2 g/L of PVP was chosen as the optimum.

Analytical Figures of Merit

The linear range, detection limit and reproducibility of the method were evaluated under the optimum





Figure 6. a) Absorbance spectra of Ag-NPs in the presence of different concentrations of ascorbic acid (the calibration curve for ascorbic acid is shown in the inset figure). b) Color images of the glass bottle containing Ag-NPs formed in the presence of different concentrations of ascorbic acid.

conditions described above. Figure 6 shows the spectra of the Ag-NPs formed upon treatment with different concentrations of ascorbic acid.

As the concentration of the ascorbic acid increases, the absorbance corresponding to the plasmon of the Ag-NPs is intensified. Under the specified experimental conditions, the calibration curve for ascorbic acid was linear from $3 \times 10^{-6} - 5 \times 10^{-5}$ M. The detection limit $(3S_b)$ [25], obtained for the determination of ascorbic acid, was 8×10^{-7} M.

Application

The proposed method was successfully applied to the determination of ascorbic acid in pharmaceutical preparations. In order to assess the possible analytical applications of the proposed method, the effect of common excipients used in pharmaceutical preparations was studied by analyzing synthetic sample solutions containing 5.0×10^{-5} M ascorbic acid and a 10-fold excess of each excipient. The undissolved material, if any, was filtered before measurement. No interference was observed from any of the excipients like glucose, starch, sugar, dextrin, talc and calcium sulfate, because, in real samples analyzed, binders and excipients cannot reduce silver ions at room temperature. So, this

Samples	Ascorbic Acid Found (mg per Tablet)		
	Claimed	${\bf Proposed}~{\bf Method}^{\dagger}$	Official Method ⁺⁺
1*	250	$254.0 (\pm 4.1)$	249.2
	250	$248.5 (\pm 4.0)$	250.1
	250	$256.0 \ (\pm \ 3.8)$	251.1
2**	180	$176.0~(\pm~2.8)$	178.0
	180	$187.0 \ (\pm \ 2.9)$	181.2
	180	$181.0 (\pm 3.1)$	179.0

Table 1. Results of determination of ascorbic acid in tablet samples.

* Sample 1 was vitamin C tablet sample of Iran Daru Pakhsh,

** Sample 2 was effervescent vitamin C tablet sample of Sunlife Company, Germany,

† Mean of three replicate,

++ British Pharmacopoeia method [26].

method is free from the interference of other reducing agents. As is clear from Table 1, the results of ascorbic acid analysis by the recommended procedure agree well with those obtained by the official method [26].

CONCLUSION

The salient features of the present study, using the seed mediation production of Ag-NPs by ascorbic acid, and an optical readout based on the absorbance of Ag nanoparticle plasmons, have excellent sensitivity. Therefore, the proposed method can be used as an alternative for determination of bulk samples and various pharmaceutical formulations in microgram quantities.

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BIOGRAPHIES

Mohammad Reza Hormozi Nezhad is Assistant Professor of Analytical Chemistry in the Chemistry Department and an Associate Member of the Institute of Nanoscience and Nanotechnology at Sharif University of Technology in Iran. He earned a Ph.D. in Analytical Chemistry from Shiraz University, Iran, followed by a one year sabbatical period at the University of Alberta (Professor Jillian Buriak's group) and a post-doctoral degree at the Institute of Nanoscience and Nanotechnology at Sharif University of Technology. His research interests include: Synthesis and Application of Colloidal Metallic and Semiconductor Nanoparticles in Analytical Chemistry.

Mohammad Ali Karimi is Associate Professor in the Department of Chemistry, and the Nanoscience and Nanotechnology Research Laboratory (NNRL) at Payame Noor University (PNU) in Sirjan. He earned a Ph.D. in Analytical Chemistry from Shiraz University, Iran. His research interests include: Application of Magnetic Nanoparticles in Solid Phase Extraction, Synthesis and Application of Metal Oxide Nanoparticles in Analytical Chemistry.

Fatemeh Shahheydari obtained her B.S. degree in Pure Chemistry from The University of Guilan, and her M.S. degree in Analytical Chemistry from Payame Noor University (PNU) in Sirjan, Iran.