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# Colorimetric sensing of cysteine using label-free silver nanoparticles

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ABSTRACT

The development of methods for the effective measurement of cysteine in biological fluids is of great physiological and pathological importance. In this study, we present a convenient and economical colorimetric method for the quantitative determination of cysteine using label-free silver nanoparticles (Ag NPs) as color indicators and $Ca^{2+}$ ions as cross-linking agents. The simultaneous addition of cysteine and $Ca^{2+}$ to a Ag NPs colloidal solution results in the cross-linking of the Ag NPs, leading to a rapid yellow-to-red color change. In this way, a simple and rapid method for the quantitative assay of cysteine in the linear range of 0.1–1000 μM was obtained through monitoring of absorbance signatures. This convenient technique exhibited high selectivity toward cysteine over a range of other amino acids, as well some thiol-containing compounds. Importantly, our newly developed method was successfully applied to the detection of cysteine in biological fluids, such as serum and artificial cerebrospinal fluid based on changes in the color of these solutions in less than 10 min.



## 1. Introduction

Cysteine is a thiol-containing amino acid that plays a critical role in a variety of important cellular functions and physiological processes, and has been implicated in a range of diseases, including heart disease, sepsis, HIV, rheumatoid arthritis, acquired immune deficiency syndrome (AIDS), Parkinson's disease and Alzheimer's disease [1–5]. Given the fundamental role of cysteine in biological systems, the development of methods for the accurate identification and quantification of this amino acid in biological samples is of critical importance. Although a variety of methods have been developed for the detection of cysteine, including high-performance liquid chromatography [6,7], fluorescence spectroscopy [8–11], capillary electrophoresis [12,13] and electrochemical voltammetry [14–16], some of these methods are expensive, complex, time-consuming, and low throughput, which limited the scope of these methods in terms of their practical application. With this in mind, there is an urgent need for the development of a simple and rapid sensor capable of identifying cysteine with high sensitivity and specificity.

Following recent developments in nanotechnology, colorimetric sensing based on metallic nanoparticles has emerged as a promising technique for biochemical analysis because systems of this type provide a simple readout (often visible with the naked eye), as well as being highly sensitive and low cost [17,18]. Among all of the materials explored in the colorimetric assay, gold nanoparticles (Au NPs) and silver nanoparticles (Ag NPs) have received considerable attention because of their surface plasmon resonance (SPR) characteristics, chemical stability, and ease of preparation. Furthermore, the surfaces of these Ag and Au NPs can be readily tailored through synthesis. By virtue of their unique optical properties (i.e., their prominent color changes determined by size-dependent SPR absorption), surface functionalized Au and Ag NPs have been designed as colorimetric probes for the detection of a broad range of analytes, including DNA, proteins, small molecules, and metal ions [19–22]. Significant advances have recently been made in the development of colorimetric sensors for the detection of cysteine. Mirkin's groups reported a colorimetric sensor for the detection of cysteine involving oligonucleotide-functionalized Au NP probes based upon T(thymidine)-$Hg^{2+}$-T coordination and the thiophilicity of $Hg^{2+}$ [23]. Chen et al. [24] designed an optical sensor for cysteine based on the cysteine-mediated aggregation of the ssDNA stabilized Au NPs in the presence of salt. Weia et al. [25] used carboxymethyl cellulose-functionalized Au NPs for the colorimetric detection of cysteine, and reported a detection limit of 100 nM. For colorimetric assays, there are several advantages to using Ag NPs over Au NPs because they possess higher extinction coefficients relative to Au NPs of the same size and are relatively low-cost [26]. Sodium dodecyl sulfate capped Ag NPs have recently been used as

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colorimetric sensors for the detection of cysteine [27]. However, the existing procedures for the fabrication of colorimetric sensors usually involve the introduction of responsive functional groups to the surfaces of the NPs through chemical modification, and extensive purification procedures are generally required to separate the modified nanoparticles from the unmodified starting materials and surplus ligands. These additional steps can be time-consuming and costly. Besides, systems of this type achieving a selective response in a complex matrix or a real biological sample has not yet been reported.

As a part of our ongoing studies toward the development of metallic nanoparticle-based colorimetric sensors [28–31], we herein describe the development of a simple and robust colorimetric method capable of sensing cysteine with high selectivity and sensitivity using label-free Ag NPs as a color indicator and $Ca^{2+}$ as a cross-linker. This newly developed colorimetric system is based on the strong interaction between the thiol group of cysteine and silver, as well as the coordination chemistry between cysteine and $Ca^{2+}$ ions. Firstly, the thiol group of cysteine interacts with the Ag NPs through the formation of an Ag—S bond. The cysteine analyte on the surface of the Ag NPs then forms a complex with the $Ca^{2+}$ ions, with a ratio of two cysteine residues to each $Ca^{2+}$ ion [32–34]. The $Ca^{2+}$ ions then bridged the nanoparticles through complexation with the cysteine residues and induced the aggregation of the nanoparticles, which resulted in appreciable changes in the color and absorption properties. In this way, we have successfully developed a sensitive colorimetric assay for the quantification of cysteine. This newly developed assay is highly selective and free from the interference of other natural amino acids and other thiol-containing compounds, as well as the species commonly found in biological fluids such as ions, glucose, and ascorbic acid. This strategy overcomes the low selectivity issues of previously reported unmodified-NP responsive systems, as well as pollution problems resulting from heavy metal ions in cysteine sensing [35–37]. Importantly, the selective colorimetric detection of cysteine in a complex matrix using Ag NPs-based system was first successfully demonstrated in the work.

## 2. Materials and methods

### 2.1. Chemicals

Sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), tri-sodium citrate and all of the amino acids used in the current study were obtained from Beijing Chemicals Ltd. (Beijing, China). Bovine serum albumin, glucose, ascorbic acid, calcium chloride ($CaCl_2$) and the other metal salts used in the current study were purchased from Shanghai Chemical Factory (Shanghai, China). All of the chemicals were purchased as the analytical-grade and used without further purification. The deionized and distilled water used in the experiments were obtained from a Mill-Q system (Millipore Corp., Bedford, USA). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used to adjust the pH.

### 2.2. Synthesis of Ag nanoparticles (Ag NPs)

All of the glassware used in this experiment was thoroughly washed with fresh water before being washed with Mill-Q water and air dried. The Ag NPs were prepared according to a modified version of a previously reported procedure [38]. Briefly, a solution of 1% tri-sodium citrate (2 mL) was added to 0.3 mM silver nitrate solution (100 mL), and the resulting mixture was stirred for 5 min. A 1 mM solution of sodium borohydride (5 mL) was added to the reaction mixture in a drop-wise manner in the absence of light, and the resulting mixture was stirred for 2 h at room temperature. The bright yellow Ag NPs were then filtrated through a Millipore syringe (0.45 μm) to remove the precipitate, and the filtrate was collected and stored in a refrigerator at 4 °C prior to its use.

### 2.3. Characterization

The Ag NPs were characterized by transmission electron microscopy (TEM) and UV–vis spectroscopy. The UV–vis spectroscopy measurements were performed on a T6 series UV–vis spectrophotometer (Puxi Co., Beijing, China). The morphologies of the Ag NPs were observed on an FEI Tecnai G2 Spirit Bio TWIN electron microscope (FEI Company, Eindhoven, The Netherlands). The samples were dropped onto a small copper mesh and left at room temperature to allow the samples to precipitate in a homogeneous manner on the carbon films between the tiny pores of the copper mesh.

### 2.4. Sample preparation

Cysteine ($10^{-2}$ M) was used as a stock solution and was serially diluted to give cysteine solutions with lower concentrations. All of the metal salt solutions used for the experiments were prepared by mixing the requisite amount of salt in water. The artificial cerebrospinal fluid (aCSF) sample containing 119 mM NaCl, 26.2 mM $NaHCO_3$, 2.5 mM KCl, 1 mM $NaH_2PO_4$, 2.5 mM $CaCl_2$, 1.3 mM $MgCl_2$, and 10 mM glucose was prepared immediately before the experiment by mixing the requisite amount of compounds into Milli-Q water. To eliminate any interference from the highly concentrated salts in the aCSF sample, the material was diluted 2-fold with water to prepare cysteine solutions with different concentrations prior to each measurement. For the detection of serum samples, the serum was diluted 20-fold with water to prepare cysteine solutions.

### 2.5. Detection of cysteine

The Ag NPs solution (20 mL) was initially diluted with a 5 mM phosphate buffer solution (80 mL, pH 7.4) for cysteine detection. In a typical sample assay, samples (0.2 mL) containing different concentrations of cysteine were added to the Ag NPs suspension (1.6 mL) followed by addition of 5 mM $Ca^{2+}$ ions (0.2 mL). The resulting mixtures were allowed to react for 8 min at room temperature, and the absorption spectra and photographs of the reaction mixtures were then recorded with a UV–vis spectrometer and camera, respectively. The ratio of the absorption at 545 nm to that at 393 nm ($A_{545}/A_{393}$) was used to quantify the amount of cysteine in the system. The selectivity for cysteine was confirmed by adding other amino acids instead of cysteine.

### 2.6. Recovery experiments

The recovery experiments were performed at the above-mentioned conditions using cysteine-spiked 20-fold diluted aCSF samples. The absorption measurements were performed using a UV–vis spectrophotometer to obtain the absorption ratio ($A_{545}/A_{393}$). Then, using the absorption ratio ($A_{545}/A_{393}$) and linear regression equations, we calculated the cysteine concentration in the samples. Finally, recovery values were calculated using the following formula:

$$\text{Recovery}\,(\%) = \frac{\text{calculated cysteine}}{\text{added cysteine}} \times 100\%$$