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Selective determination of cysteine by resonance light scattering of silver nanoparticles

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Abstract: A novel sensitive method has been developed for the selective determination of cysteine in shampoo and pill food by using resonance light scattering (RLS). The interaction between cysteine and silver nanoparticles was studied. Through the covalent combination with the –SH group and the electrostatic binding with the $-NH_3^+$ group of cysteine, silver nanoparticles can self assemble to form a network structure, which results in greatly absorption signal. The experimental result showed that the RLS technique offers a sensitive method for investigations of self–assembly of nanoparticles. The linear range is 0.01 to 0.31 mg L^{-1} and the limit of detection (LOD) is 5×10^{-3} mg L^{-1} for determination of cysteine. The relative standard deviation (RSD) was 4.1% for cysteine concentration of 0.10 mg L^{-1} (n=5). The method was successfully applied for determination of cysteine in some real samples which gave satisfactory results.
Keywords: RLS; Silver nanoparticles; cysteine.

1. Introduction

Nowadays the nanoparticles are the most popular elements in the nanoscience and its technology due to their various applications in the chemical, electronic, agricultural, pharmacy and medical industries. Based on the chemical compound type, the nanoparticle to be divided to various sorts, such as metalic, cermical, polymerical and semiconductive nanoparticles.

Cysteine plays an important role in several biological processes, and many important cellular functions, such as detoxification and metabolism [1]. Determination of cysteine or compounds of cysteine are commonly used in clinical investigation, pharmaceutical industry, and research. Many methods for its determination have been reported including high-performance liquid chromatography (HPLC) [2, 3, 4], or gas–chromatography methods [5, 6], which was very popular with equipment easily available in many laboratories, but several disadvantages can be cited. In all of chromatographic methods, the samples should pass through derivatization and extraction of products of reaction before their microinjection the column. Those methods were used expensive reagents and equipment, and a significant period of time for whole assay. The other methods are chemiluminescence [7, 8], capillary electrophoresis [9], electrochemistry [10], and fluorimetry [11], that based on the redox chemistry or derivatization with chromophores or fluorophores. Recently an entirely new colorimetric detection strategy for cysteine, based on the color change originated from the cysteine–directed self–assembly of gold nanoparticles and gold nanorods, was reported [12, 13, 14], the detection strategy has offered significant advantages of rapid procedure and no requirement of expensive instrumentation. However

the main disadvantage of these approaches is their low sensitives in which only micromolar concentration of cysteine detectable. Herein, we report the sensing of cysteine using resonance light scattering (RLS) based on self-assembly of silver nanoparticles absorption. In the present method, silver nanoparticles can self–assemble to form a network structure. The large size of assemble of the silver nanoparticles and the surface plasmons coupling among silver nanoparticles lead to strong absorption. The sensitivity increases about two orders of magnitude that of colorimetric methods. All substances found in samples do not interfere with determination of cysteine, the results indicating that the proposed method has a good selectivity.

2. Experimental

2.1 Apparatus

The absorption spectra were measured by a Lambda 25 UV-Vis spectrometer (Perkin Elmer, Germany). The TEM images of the silver nanoparticles were acquired on a JEM-l00SX transmission electron microscope (Tokyo, Japan) and the pH values were measured with a pHs-3C Precision pH meter (Metrohm, Swiss). A MVS-1 vortex mixer (Heidolph Instruments, Germany) was used to blend mixtures.

2.2 Reagents

Trisodium citrate, silver nitrate, cysteine and all of other chemicals were purchased from Merck (Germany). The stock solution was prepared by dissolving appropriate weight amount of cysteine in water. The working solutions were prepared by serially dilution of the stock solution. Doubly distilled water was used throughout the experiment.

2.3 Sample preparations

Shampoo: First, a 0.20 mL of shampoo was transferred to a 10.00 mL volumetric flask and the volume was made up with doubly distilled water, then 0.1 mL of the diluted solution was transferred to a test tube and the volume was adjusted to 1 mL with acetate buffer at pH 5.0.

Pill food: We ground a pill by mortar and dissolved it in 100 mL of doubly distilled water. A 0.10 mL of this solution was transferred to a test tube and adjusted to 1 mL with acetate buffer at pH 5.0.

2.4 Preparation of silver nanoparticles

Nowadays nanoparticles are making from various compounds. The most common nanoparticles are ceramical nanoparticles, metallic, polymerical and semiconductive nanoparticles. In this paper we prepared silver nanoparticles according to slightly modified Lee and Meisele method based on the reduction of silver salt by citrate [15]. The method is called chemical reduction. In 250 mL of doubly distilled water, 45 mg of silver nitrate was dissolved and this solution was heated to boiling temperature, then different value of a 1% trisodium citrate aqueous solution was added drop by drop into the boiling silver nitrate solution to making various particles size and accompanied by vigorous stirring simultaneously. The mixed solution was kept boiling for another 10 min. Finally, a pale yellow silver colloid was obtained. Then it was removed from heating element and stirred until reaching to room temperature. The reaction could be expressed as follows [16]:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow$$

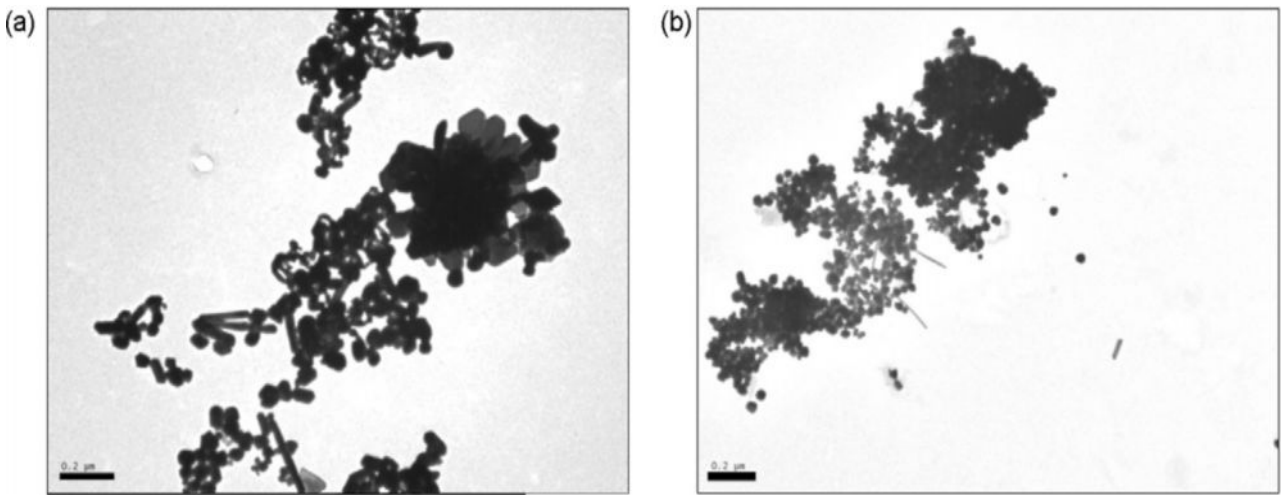

Fig. 1. TEM images of Ag colloids with: (a) 2.5 mL and (b) 10 mL citrate concentration.

2.5 Effect of citrate concentration

For studying the influence of citrate concentration on the surface plasmon resonance (SPR) and its absorption, colloids consisting of different particles size were prepared by adding 2.5, 5.0 and 10.0 mL of citrate to boiling silver nitrate solution. Solutions of colloidal Ag nanoparticles had distinctive color arising from their tiny dimensions [17], Fig. 1. is showing two shapes of Ag colloids formed by addition 2.5 and 10 mL of citrate concentration.

3. Results and discussion

3.1 Characteristic of the absorption spectra

The optical absorption spectrum of metal nanoparticles is dominated by SPR which exhibits a shift towards the red end or blue end depending on particle size, shape, state of aggregation and the surrounding dielectric medium. The absorption bond in the visible light region is typical for silver nanoparticles. The plasmon peak depends on the extent of colloidal aggregation. Since the colloidal silver particles possessed a negative charge due to the absorbed citrate ions, a repulsive force worked along particles and prevents aggregation. Colloid for which, maximum SPR intensity and wavelength represents a broad absorption band, with SPR at 453 nm. The broader absorption band is an indication of larger particles size. Citrate can be used as both reducing agent and stabilizer of the colloidal particles formed, since it exerts a drastic effect on size and size distribution of nanoparticles prepared under constant conditions. It is difficult to distinguish between the reducing and stabilizing actions of citrate, since a variation in its concentration may cause both a change in the reduction rate and in the nucleation–to–growth ratio [18]. The quantity of citrate is varied from 2.5 to 10 mL, it is found that the absorption wavelength shift towards shorter wavelength region, which is characteristic for a decrease in particle size from 75 nm to approximately 16 nm.

Fig. 2 shows the absorption spectra the conjugates of silver nanoparticles with different concentration of cysteine in the wavelength range 300-800 nm. Silver nanoparticles exhibit one absorption peak at 453 nm. This peak is greatly enhanced when a small amount of cysteine is added into the solution. The diameter of silver nanoparticles is approximately 16 nm. With increase the cysteine concentration the absorption intensity will be increases. According to the resonance light scattering theory, the RLS effects is observed as increased scattering intensity at or very near to the wavelength of absorption of an aggregated molecular species [19].

The absorption can be dramatically enhanced when cysteine add to nanoparticles solution. Pale yellow color of the silver nanoparticles became a bit darker when a small amount of cysteine is added into the solution. When silver nanoparticles aggregate and the interparticle distance in aggregates decreases to less than approximately the average particle diameter, the color of the aggregates tunes a bit darker which result in the shift of absorption band to longer wavelengths because of the dipole-dipole interaction and coupling between the plasmons of neighboring particles in the formed aggregates [20]. It can be concluded that the assembly of silver nanoparticles and the interparticles plasmons coupling result in great enhancement of absorption of silver nanoparticles. On the other hand, the assembly of silver nanoparticles via cysteine leads to the size increases of the absorption, which results in the absorption enhancement in all of the wavelength range from 300 to 800 nm. There are a mercapto group and an amino group in cysteine. Cysteine can combine with the silver nanoparticles through Ag-S covalent bond. The amino group in cysteine is a positively charged group at pH=5.0, which integrates with the negative charge on the surface of other silver nanoparticles through electrostatic binding. As a result, the silver nanoparticles assemble each other via cysteine and form a network structure.

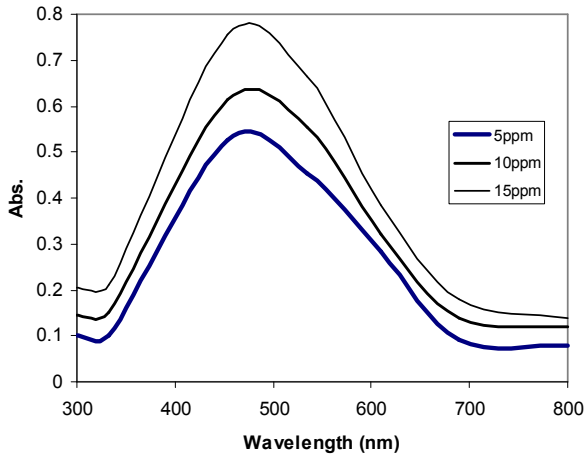

Fig.2. Light scattering spectra of silver nanoparticle solutions in presence of cysteine.The concentrations of cysteine are (a) 5 mg L^{-1}, (b) 10 mg L^{-1}, (c) 15 mg L^{-1}.

3.2 Effect of pH

The influence of solution pH on interaction between silver nanoparticles and cysteine was studied over the pH range from 3.0 to 7.0, because the pH of the solution plays an important role on amount of the absorption intensity. As shown in Fig. 3, the absorption intensity of silver nanoparticles–cysteine conjugates greatly depends on the solution pH with maximum value at pH 5.0. Increasing pH of the solution caused the absorption intensity of silver nanoparticles-cysteine increases slowly. The amino group is positively charged in our experimental pH range. Therefore, with increasing pH, the ionization of the –COOH group in citrate on silver nanoparticles surface increases, then with increasing pH value, the electrostatic biding between the $-NH_3^+$ group and the $-COO^-$ group become stronger. As the pH values increase, the absorption intensity of the system increases. Ionization of the other hydrogen ions of citrate on the silver nanoparticles surface is almost complete at pH 5.0. Therefore, the absorption intensity of silver nanoparticles–cysteine conjugates reaches maximum at pH 5.0. The increasing ionic strength of the solution also induces the aggregation of silver nanoparticles, which results in the increase of absorption intensity of the system [21].

3.3 Effect of buffer concentration

We showed the influence of buffer concentration on absorption intensity in Fig. 4. Upon increasing buffer concentration, the absorption intensity of silver nanoparticles–cysteine conjugates reaches the maximum at 0.10 M of buffer concentration, and then gradually decreases with increasing buffer concentration. The result indicates that a high ionic strength can destroy the electrostatic binding between the $-NH_3^+$ group and the $-COO^-$ group of citrate on the silver nanoparticles surface. Therefore, we used a 0.10 mol L^{-1} acetate buffer with pH 5.0 to adjust solution pH.

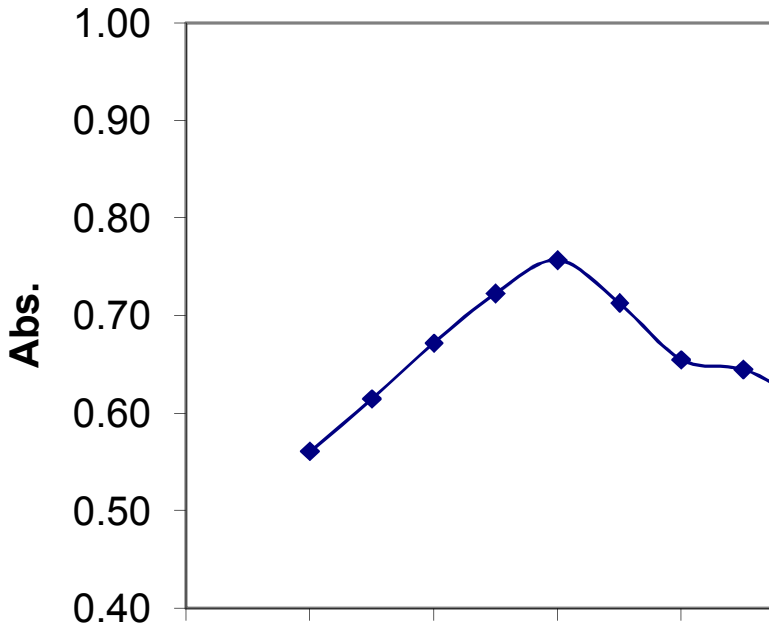

Fig. 3. Effect of pH on the absorption signal of silver nonoparticles-0.2 mg L^{-1} cysteine. The pH values of Solutions were controlled by acetate buffer with different concentration ratio of acetate in the pH range 3.0-7.0.

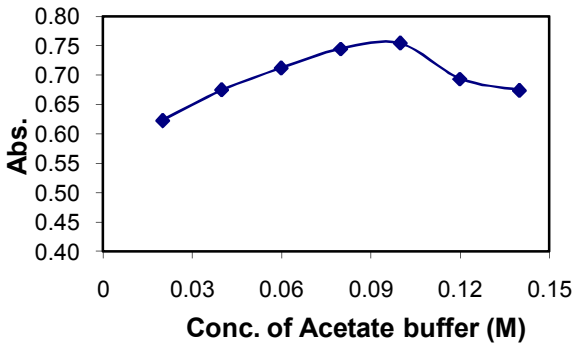

Fig. 4. Influence of buffer concentration on absorption intensity of silver nonoparticles-0.2 mg L^{-1} cysteine. The concentration of acetate in the buffer solution was varied from 0.02 to 0.14 M (pH 5.0).

3.4 Study the silver nanoparticles concentration

Fig. 5, showing optimization curve for silver nanoparticles concentration. The absorption of silver nanoparticles-cysteine conjugates greatly increases when the concentration of silver nanoparticles solution is varied from 0.1 to 0.7, then decreases beyond 0.3 until 0.7. The absorption intensity of solution at silver nanoparticles concentration of 0.4 becomes lower than that at silver nanoparticles concentration of 0.3, Therefore, the concentration of silver nanoparticle of 0.3 chosen as being optimum for subsequent work. Briefly, for preparation of silver nanoparticle in different size, after weighting different mass of silver nitrate and dissolving them in boiling water, 5 mL of 0.1% trisodium citrate solution was added drop by drop into the boiling solutions and accompanied by vigorous stirring.

3.5 Stability of absorption intensity (influence of incubation time)

Table. 1, showed the influence of incubation time on absorption intensity of silver nanoparticles cysteine conjugates. The influence of incubation time on absorption was investigated in period of 120 min immediately after mixing the cysteine and silver nanoparticles in mentioned buffer solution at pH 5.0. The absorption intensity of silver nanoparticles–cysteine conjugates reaches a plateau in 60 min and is stable for at least 120 min. The stabilization datas showing that solution of silver nanoparticles at pH 5.0 is stable at least 120 min. Therefore, the stability of the absorption intensity is critical for cysteine determination.

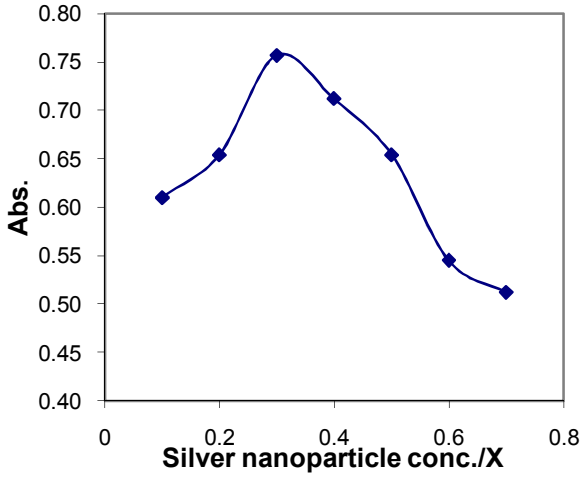

Fig. 5. Effect of silver nanoparticles concentration on the absorption intensity of silver nanoparticles 0.2 mg L^{-1} cysteine.

Table 1:Stability of absorption signal of silver nanoparticles-0.2 mg L^{-1} cysteine. (Acetate buffer of 0.10 M)

Time(min)	Abs.
00	0.438
20	0.533
40	0.683
60	0.757
80	0.756
100	0.758
120	0.757

3.6 Determination of cysteine

Under the optimized conditions mentioned above, the calibration curve was gotten over the cysteine concentration range of 0.01 to 0.31 mg L^{-1}. There was a good linear relationship between the concentration of cysteine and absorption intensity. The regression equation was Y=0.4596+1.4423C where Y is absorbance and C is concentration of system (mg L^{-1}), and corresponding regression coefficient was R^2=0.9965, relative standard deviation was 1.4 % at cysteine concentration of 0.100 mg L^{-1} (n=5).

3.7 Applications

This method using silver nanoparticles was applied to determination of cysteine in the shampoo and pill food samples. As previous mentioned procedure to preparation of sample, we measured the absorption of cysteine in the mentioned sample according to standard addition method. The results are listed in table. 2.

Table 2:Determination of cysteine² in shampoo and pill food

Sample	added (mg L^{-1})	found (mg L^{-1})	Recovery (n=5)
Shampoo			
1	0.0	0.03	-
2	0.01	0.04	97 %
3	0.05	0.09	100 %
Pill food			
1	0.0	0.05	-
2	0.01	0.16	100 %
3	0.05	0.14	99 %

Conclusion

A novel method for determination of cysteine has been developed based on the self–assemble of the silver nanoparticles to form a network through the covalent combination with the -SH group and electrostatic binding with the $–NH_3^+$ group of cysteine. This phenomenon results in greatly enhanced absorption intensity. The proposed method is simple and sensitive for determination of cysteine and offers high selectivity because only cysteine contains both the –SH and $–NH_3^+$ groups among all kinds of substances found in our real sample. Moreover, this method had a satisfactory sensitivity and can be applied directly to determination of cysteine in the shampoo and pill food without any hard interfering or even separation.

Acknowledgments

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