



## Visual colorimetric detection of berberine hydrochloride with silver nanoparticles

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### ABSTRACT

A visual colorimetric method for the detection of berberine hydrochloride was proposed in this contribution based on the color change caused by the aggregation of silver nanoparticles (AgNps). It was found that citrate-capped AgNps dispersed in water owing to the electrostatic repulsion from each other by the negative charged surface, presenting a bright yellow color. However, the presence of positively charged berberine could induce the aggregation of citrate-capped AgNps, resulting in color change from yellow to green and even to blue depending on the concentration of berberine. Under the optimum condition investigated with UV–vis absorption and light scattering technique, berberine hydrochloride from 0.05  $\mu\text{mol l}^{-1}$  to 0.4  $\mu\text{mol l}^{-1}$  could be visually detected based on the color alteration of the AgNps suspension.

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### 1. Introduction

Berberine (Fig. 1) is an important anti-inflammatory drug for heart and intestinal disorders widely, owing to its antitumor promotion active and anti-lipase effective [1]. The quantification of berberine is usually required in clinic medical assay. Analytical methods for the detection of berberine have been established including spectrophotometry [2], fluorescence spectrometry [3], light scattering spectrometry [4,5] and optical fiber sensing [6]. These methods have advantages of high sensitivity and selectivity, but require expensive instruments or complicated procedures.

Metal nanomaterials such as silver nanoparticles (AgNps) have been found wide applications for analytical purposes owing to their unique optical and electric properties. The applications of AgNps in analytical chemistry, however were largely focused on the field of surface-enhanced Raman scattering (SERS) since the discovery of much intense Raman scattering signals from the molecules adsorbed on the surface of AgNps [7,8]. Owing to the collective oscillations of conducting electrons under the excitation of a light beam [9] known as surface plasmon resonance (SPR) which depends on

the size, shape and dielectric environment of nanoparticles, AgNps have bright color in the visible spectral range concerning their SPR absorption and light scattering [10,11]. Therefore, the applications of AgNps in the field of surface plasmon resonance spectroscopy [12,13], colorimetric detection [14,15] and scattering detection [16] have been stimulated in recent years.

Colorimetric sensor is very useful in the analysis of biomolecular and metal ions [17–27] for its simplicity, rapidity and not involving in any complicate apparatus. For example, two batches of 13-nm gold nanoparticles bounded with non-complementary DNA oligonucleotide-capped with thiol groups, can self-assemble into aggregation upon the addition of an oligonucleotide duplex complementary to the two grafted sequences [28]. This strategy has made it possible to tune the optical properties of colloidal gold, and establish a colorimetric sensor for polynucleotides [17,18,29,30]. Another example is that Rothberg proposed a colorimetric method for hybridization assay [19] based on the findings that single- and double-stranded oligonucleotides have different propensities to be adsorbed on the surface of gold nanoparticles in colloidal solution. Other colorimetric methods were also been established and used in the detection of proteins [31] and metal ions [32,33].

Citrate-capped AgNps have electronegative charged surface and can be dispersed from each other in the water symmetrically by the electrostatic repulsion, exhibiting yellow color for the plasmon resonance absorption [34,35], however, the presence of positive charged berberine would induce the aggregation of AgNps owing to

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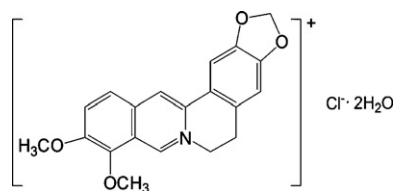


Fig. 1. Molecular structure of the berberine hydrochloride.

the electrostatic attraction, causing a color change of AgNps suspension. This strategy allows us proposing a visual colorimetric method for the detection of berberine based on the aggregation of AgNps in this contribution. It was found that the colloid of AgNps would change from yellow to green and even to blue depending on the concentration of berberine. Therefore, the colorimetric detection of berberine can be established by visualizing the color of AgNps suspension by naked eye without any equipment.

## 2. Experimental

### 2.1. Apparatus

The absorption spectra of AgNps were measured on a Hitachi UV–vis 3010 spectrophotometer (Tokyo, Japan) with a 10-mm path length quartz cuvette. The light scattering spectra were obtained with a Hitachi F-4500 spectrofluorometer (Tokyo, Japan) equipped with a 150-W Xe lamp by simultaneously scanning the excitation and emission monochromators without wavelength difference. SEM image of AgNps was captured with the Hitachi S-4800 scanning electron microscopy (Tokyo, Japan). Photographs of the AgNps suspension used for visual colorimetric detection were captured by a Nikon 4500 digital camera. A pH-3C digital pH meter (Xiaoshan Scientific Instrument Plant, Zhejiang, China) was used to measure the pH values of the aqueous solutions, and MVS-1 vortex mixer (Beide Scientific Instrument Ltd., Beijing, China) was used to blend the solutions in volumetric flasks. SZCL-3A digital controlled magnetic stirring hotplate (Gongyi Yuhua Instrumental Ltd., Henan, PRC) was used to prepare AgNps.

### 2.2. Reagents

A  $1.00 \times 10^{-3} \text{ mol l}^{-1}$  stock solution of the hydrochloride salt of berberine (E. Merck, Darmstadt, Germany) was prepared by directly dissolving the commercially purchased product into super-purified water (18.2 M $\Omega$ , LD-50G-E Ultra-Pure Water System, Lidi Modern Waters Equipments Co., Chongqing, China), and then diluted to  $1.0 \times 10^{-5} \text{ mol l}^{-1}$  as a working solution.  $0.1 \text{ mol l}^{-1}$  HCl, and  $0.1 \text{ mol l}^{-1}$  NaCl were used to adjust the acidity and ionic strength. 1% (w/w) trisodium citrate was used as a stabilizer in the preparation of AgNps. All reagents used in this experiment were of analytical grade without further purification, and super-purified water (18.2 M $\Omega$ ) was used throughout the experiment.

### 2.3. Preparation of silver nanoparticles

The AgNps were prepared with modified Creighton method [36], which employed citrate sodium as a stabilizer capped on the AgNps surface [34]. Shortly, 50 ml solution of AgNO<sub>3</sub> with final concentration of  $0.25 \text{ mmol l}^{-1}$  was first prepared in the ice bath. Under vigorously magnetic stirring, 1 ml 1% trisodium citrate and  $0.6 \text{ ml } 10 \text{ mmol l}^{-1}$  NaBH<sub>4</sub> was added to the solution, respectively. The stirring was stopped after 45 min, and the prepared AgNps suspension could be used after 2 h. After characterized with UV–vis absorption and scanning electron microscopic measurements, the AgNps sus-

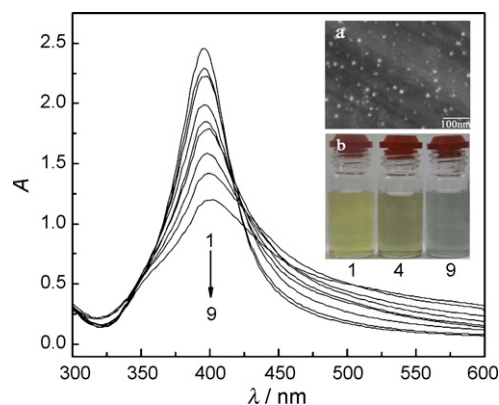


Fig. 2. SPR absorption of AgNps in the presence of berberine hydrochloride. Condition:  $c_{\text{Ag}}$ ,  $2.5 \times 10^{-4} \text{ mol l}^{-1}$  (calculated with silver atom);  $c_{\text{berberine}}$  (from 1 to  $9 \times 10^{-7} \text{ mol l}^{-1}$ ), 0, 1, 1.5, 2, 3, 3.5, 4, 4.5, 5; pH, 3.42;  $c_{\text{NaCl}}$ ,  $6 \text{ mmol l}^{-1}$ , standing time, 5 min. The inserted are typical SEM image of AgNps (a) and photos of AgNps suspension (b). (For interpretation of the references to color in text, the reader is referred to the web version of the article.)

pension was stored in darkroom allowing its stability for several weeks.

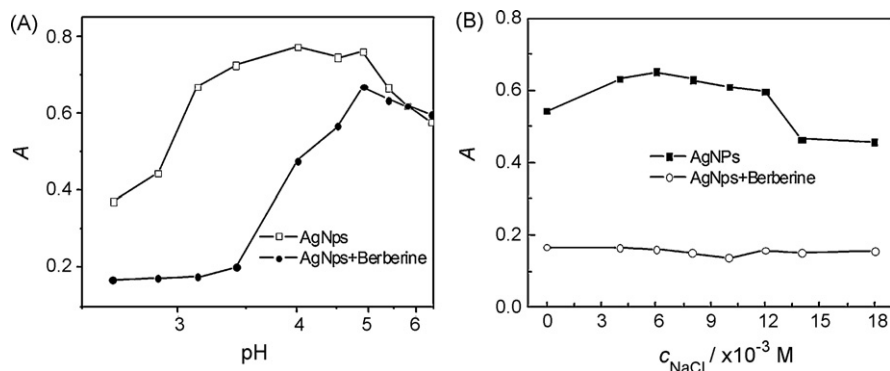
### 2.4. Procedures

Typically, 1 ml of prepared  $2.5 \times 10^{-4} \text{ mol l}^{-1}$  AgNps suspension, 0.90 ml of  $0.1 \text{ mol l}^{-1}$  HCl, 0.30 ml of  $0.1 \text{ mol l}^{-1}$  NaCl and berberine hydrochloride were successively added into a 10-ml calibrated tube. The mixture in the calibrated tube was vortexed after each addition of the interacting additives, and diluted to 5.0 ml with thorough mixing after 5 min. Then the solution was transferred into a 10-mm optical quartz cell for the measurements of the UV–vis absorption and light scattering properties on UV–vis spectrophotometer and spectrofluorometer, respectively. In the colorimetric detection, 2.0 ml of pH and ionic strength adjusted AgNps suspension was firstly pipetted to a 2.5-ml glass tube, and then 0.10 ml of berberine sample solution was mixed with the AgNps suspension. The photographs were captured with a digital camera 5 min later.

## 3. Results and discussion

### 3.1. Interaction of AgNps and berberine

Typically, colloidal solution of silver nanoparticles is yellow owing to its SPR absorption band extended from 400 nm to 600 nm. This SPR band is not only dependent on the size of the particle and the refractive index of the surrounding media, but also gets significant changes with shape, and inter-particle distance [35–37]. If aggregation of AgNps gets accrued, the SPR absorption band would change obviously for the decreasing of inter-particles distance, causing color change of AgNps solution. Since the citrate-capped AgNps prepared in this contribution are electronegative charged, and dispersed from each other symmetrically by the electrostatic repulsion of each particle, the aggregation of AgNps occurred when the electropositive charged berberine is mixed with the colloidal solution of AgNps owing to the neutralization of the electrostatic repulsion from each silver nanoparticle. Fig. 2 shows the SPR absorption spectra and photographs of AgNps colloidal solution with increasing berberine content. It can be seen that the SPR absorption band of AgNps suspension is centralized at 396 nm, and gets decreased obviously with increasing berberine, and the color of AgNps solution changes from yellow to green, and then even to blue depending on the berberine concentration.

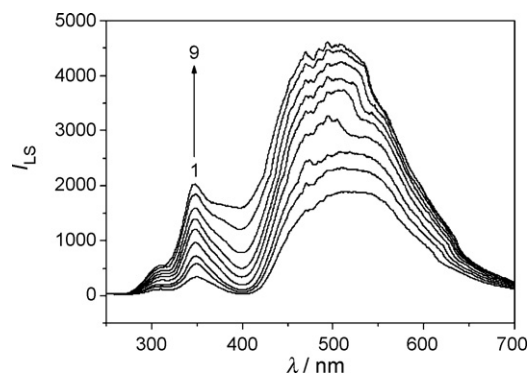


**Fig. 3.** (A) Effect of acidity of AgNps suspension. Concentration:  $c_{\text{Ag}}$ ,  $5.0 \times 10^{-5} \text{ mol l}^{-1}$ ;  $c_{\text{Berberine}}$ ,  $4.0 \times 10^{-7} \text{ mol l}^{-1}$ ;  $c_{\text{NaCl}}$ ,  $6 \text{ mmol l}^{-1}$ , standing time, 5 min (B) Effect of ionic strength on the aggregation of AgNps. Concentration:  $c_{\text{Ag}}$ ,  $5.0 \times 10^{-5} \text{ mol l}^{-1}$ ;  $c_{\text{BH}}$ ,  $4.0 \times 10^{-7} \text{ mol l}^{-1}$ , pH, 3.42, standing time, 5 min.

### 3.2. Depending factors of colorimetric detection

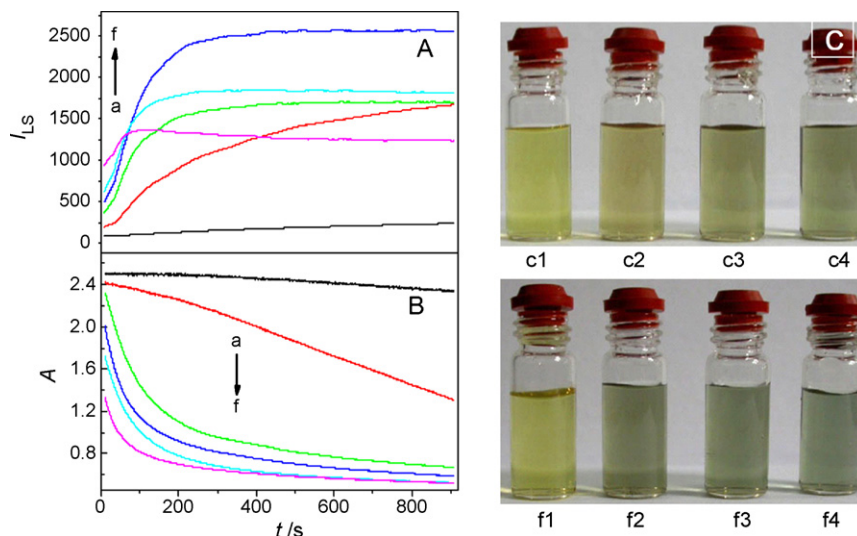
In order to make successful colorimetric detection, we should consider the factors related to color change since the color of AgNps suspension depends on the size and the surroundings. With the increase of nanoparticles size, the AgNps suspension gets changed from yellow to brown, and finally become a turbid solution. We made a comparison of Creighton method [38], which is based on the reduction of silver nitrate by sodium borohydride, and Lee-Meisel method, which is based on the reduction of silver nitrate by trisodium citrate. It was found that our modified Creighton method, which employed trisodium citrate as a stabilizer capped on the surface of AgNps, could result in bright and transparent suspension of mono-distribution AgNps with small diameter. However, Lee-Meisel method results in larger size of AgNps which do not tend to be aggregated and fail to exhibit wishful color changing process in the colorimetric detection of berberine.

The aggregation of AgNps based on the electrostatic interaction between the negatively charged citrate and positively charged berberine, can be adjusted by changing the pH of the surrounding medium. As the  $\text{p}K_{\text{a}1}$  of citric acid is 3.13, thus the citrate-capped AgNps can be stabilized by the electrostatic repulsion of negative charged  $-\text{COO}^-$  group in citrate in a medium of pH higher than 3.13. Fig. 3A shows the optimization over the range from 2.64 to 6.42. It



**Fig. 4.** Time course of the aggregation of AgNps as induced by berberine hydrochloride monitored with light scattering spectra. Concentration:  $c_{\text{Ag}}$ ,  $2.5 \times 10^{-4} \text{ mol l}^{-1}$ ;  $c_{\text{Berberine}}$ ,  $4.0 \times 10^{-7} \text{ mol l}^{-1}$ ;  $c_{\text{NaCl}}$ ,  $6 \text{ mmol l}^{-1}$ ; pH, 3.42; standing time (from 1 to 9 min), 0.2, 0.5, 0.8, 1.2, 2, 2.5, 4, 6, 8.

can be seen that no significant difference is found either berberine is present or not when pH larger than 5. With the decreasing of pH less than 5, the absorption of AgNps suspension in presence of berberine gets decreasing obviously, and reaches maximum near 3.42. However, the further decreasing of pH would induce self-aggregation of AgNps even if berberine is absent.



**Fig. 5.** Time course aggregations of AgNps in the presence of different amounts of berberine in terms of light scattering (A,  $\lambda$ , 350 nm) and plasma resonance absorption (B,  $\lambda$ , 396 nm), and their photographs (C). Condition:  $c_{\text{Ag}}$ ,  $2.5 \times 10^{-4} \text{ mol l}^{-1}$ ;  $c_{\text{Berberine}}$  (from a to f,  $\times 10^{-7} \text{ mol l}^{-1}$ ), 0.5, 2, 2.5, 3, 3.5, 4;  $c_{\text{NaCl}}$ ,  $6 \text{ mmol l}^{-1}$ ; pH, 3.42; standing time of photographs (from c1 to c4 and f1 to f4) at 0, 1, 3, and 5 min, respectively. (For interpretation of the references to color in text, the reader is referred to the web version of the article.)

**Table 1**  
Determination results of berberine in tablets

Sample	Specified (mg/piece)	Found (mg/piece)	Average (mg/piece)	R.S.D. (% , n = 5).	Recovery (% , n = 5)
1 <sup>a</sup>	30	32.3, 29.3, 29.7 30.4, 31.2	30.6	3.9	101.3
2 <sup>b</sup>	30	31.7, 30.5, 32.2, 29.4, 31.5	31.1	3.6	102.4

<sup>a</sup> Berberine tablets were purchased from Sichuan Shuzhong Pharmaceutical Ltd. (Chengdu, China).

<sup>b</sup> Sichuan Mianyang Pharmaceutical Ltd., Taiji Group (Mianyang China). Experiment condition: condition:  $c_{Ag}$ ,  $2.0 \times 10^{-4} \text{ mol l}^{-1}$ ;  $c_{NaCl}$ ,  $6 \text{ mmol l}^{-1}$ ; pH, 3.42; standing time, 5 min.

The ionic strength is another factor affecting the aggregation of AgNps. Fig. 3B shows that 6 mM of NaCl is necessary in the medium. The increasing of less inorganic salt slightly increased the absorbance of AgNps in the absence of berberine, while high ionic strength medium effectively screens charge repulsion of negative charged AgNps and directly cause the self-aggregation of AgNps even if in the absence of berberine.

### 3.3. Time course of AgNps aggregations

Dynamic process of aggregation of AgNps was investigated based on the measurements of light scattering signals and SPR absorbance considering that both of them are good features for characterizing nanoparticles aggregation [4,39].

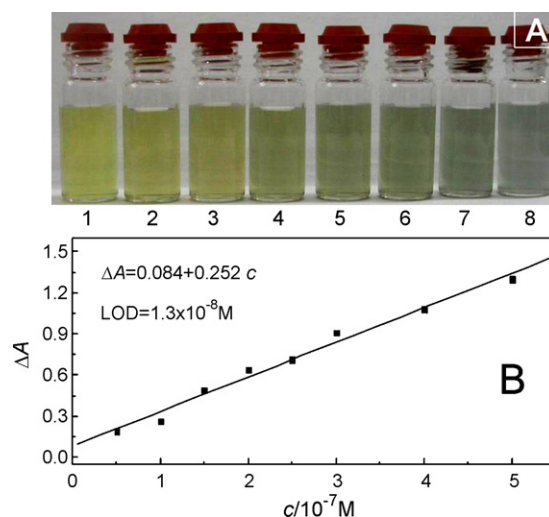
Fig. 4 shows that the light scattering spectra are characterized with two peaks localized at 347 nm and 500 nm, respectively, and their signals get increased with time within ten minutes. Further monitoring the time-dependent aggregation process of AgNps intensively in the presence of different amounts of berberine showed that the light scattering signals of the AgNps suspension get increased obviously and reach maximum in several minutes (Fig. 5A). That means the aggregation of the AgNps occurred after the addition of berberine immediately and finished in several minutes. Corresponding to the light scattering signals, this time course can also be measured based on the plasma resonance absorption. Fig. 5B shows that decreasing of SPR absorption happens with increasing berberine content.

Berberine higher than  $0.05 \mu\text{M}$  can induce the aggregation of AgNps, both SPR absorption and light scattering signals of the AgNps suspension get changed obviously in several minutes after the addition of berberine. The aggregation speed, which could be displayed with the ratio of SPR absorbance and process time, was dependent on the concentration of berberine (Fig. 5B, a–f). However, the light scattering intensity of AgNps suspension at time course is not always increased at higher berberine concentration, but decreased obviously (Fig. 5A, e and f).

Fig. 5C is the photographs of the aggregation process of AgNps at different time in the presence of berberine. In the presence of low concentration of berberine (c1–c4), the color of AgNps changes from yellow to yellow brown, and then to yellow green, while in the presence of high concentration of berberine (f1–f4), the color directly gets changed from yellow to yellow green and quickly becomes to gray blue with the final color.

### 3.4. Analytical parameter

Under the optimum condition, the colorimetric assay was processed directly using the AgNps suspension in glass tubes to detect different amounts of berberine. Fig. 6 shows the picture of AgNps solution after the interaction with seven different berberine concentrations in the range of  $0.05\text{--}0.4 \mu\text{M}$ . The color of AgNps solution gets changed as the order of yellow → dark yellow → olive → green → blue with increasing of berberine concentration (Fig. 6A). So, we can probably discriminate the concentration of berberine using naked eyes compared with the



**Fig. 6.** (A) Color ladder for the colorimetric detection of berberine hydrochloride with the common photograph of AgNps solution and (B) linear relationship between PRA absorption of AgNps suspension at 396 nm and the concentration of berberine hydrochloride. Condition:  $c_{Ag}$ ,  $2.5 \times 10^{-4} \text{ mol l}^{-1}$ ;  $c_{NaCl}$ ,  $6 \text{ m mol l}^{-1}$ ; pH, 3.42;  $c_{Berberine}$  (from tube 1 to  $8 \times 10^{-7} \text{ mol l}^{-1}$ ), 0, 0.5, 1, 1.5, 2, 2.5, 3, 4; standing time, 5 min. (For interpretation of the references to color in text, the reader is referred to the web version of the article.)

stand colorimetric picture. Furthermore, there is a good linear relationship between the absorbance change ( $\Delta A$ ) of AgNps and the concentration of berberine ( $c$ ) in the range of  $0.5\text{--}5.0 \times 10^{-7} \text{ M}$ . The standard regression equation is  $\Delta A = 0.084 + 0.252c$  and the limits of determination is  $1.3 \times 10^{-8} \text{ M}$  (Fig. 6B).

The selectivity of this method was investigated in the presence of  $0.2 \mu\text{M}$  berberine following the general procedure by premixing with potential interfering substances such as common metal ions, sugars, amino acids, and pharmaceuticals. The absorption spectra were used to monitor the aggregation of AgNps. It was found that common metal ions ( $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Fe}^{3+}$ ) and saccharides (glucose, lactose, sucrose, and maltose) can be allowed at very high concentration (50–100 times than berberine). While, common amino acids such as L-tyrosine, L-glycine, L-arginine, L-aspartic acid and L-histidine can coexist at low concentration of  $1 \mu\text{M}$ , while L-cysteine can only allowed at  $0.1 \mu\text{M}$ .

### 3.5. Pharmaceutical product detection

Berberine from pharmaceutical products were used to validate this method by measuring the absorbance of AgNps suspension. Quantification results showed in Table 1 indicate that this method is reliable and practical.

## 4. Conclusion

Colorimetric analytical method for the detection of berberine hydrochloride was established with citrate-capped AgNps. Citrate-capped AgNps with negative charged surface can be dispersed in

the water symmetrically by the electrostatic repulse interaction of each particle. However, the presence of positive berberine would induce the aggregation of AgNps electrostatic attraction, causing the color change of AgNps suspension from yellow to green, and then to blue depending on the aggregation degree of AgNps. The mechanism of color change and the effect of experiment condition were studied using absorption and light scattering spectrometry. Under the optimum condition, we can detect the berberine hydrochloride from 0.05  $\mu\text{M}$  to 0.4  $\mu\text{M}$  visually based on the color alteration of the solution. This colorimetric analytical method without use of expensive machines is so convenient, economy and speedy that it has flourishing prospects in analytical chemistry.

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