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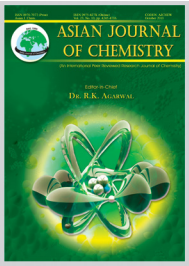

Preparation of a Chemical Sensor Based on Modified Silver Nanoparticles for Quick Recognition of 5-Fluorocytosine

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A chemical sensor of *p*-aminobenzene sulfonic acid functionalized silver nanoparticles was initially prepared, which can be used for quick recognition of 5-fluorocytosine. The presence of 5-fluorocytosine can induce the aggregation of nanoparticles due to electron donor-acceptor interaction between the target and *p*-aminobenzene sulfonic acid on the surface of silver nanoparticles, resulting in a shift in the surface plasmon band and a consequent colour change of the silver nanoparticles from yellow to green. Moreover, the high selectivity of *p*-aminobenzene sulfonic acid-modified silver nanoparticles for the target was approved with a significantly higher absorption ratio (A_{645}/A_{390}) compared with other seven molecules with similar structures. The concentration of 5-fluorocytosine was determined with a limit of detection lower than 0.08 mg/L by the naked eyes and the sensitivity could be enhanced by the adjustment of ion strength and pH.

Key Words: Silver nanoparticles, 5-Fluorocytosine, *p*-Aminobenzene sulfonic acid, Chemical sensor.

INTRODUCTION

With the recent advances in nanotechnology, nanoparticles have attracted considerable interests due to their unique photochemical, photocatalytic, photovoltaic properties, *etc.*[1-3]. The extremely small size of nanoparticles in the range of 10-100 nm means they exhibit enhanced or different properties when compared with the bulk material. In the past decades, significant advances have been made in the development and application of gold nanoparticles. In comparison, for silver nanoparticles (Ag NPs), there has been recently been interested in investigating their optical properties and application since they can also strongly absorb in the visible region due to surface plasmon resonance, possessing high surface to volume ratio and much higher extinction coefficients[4-7]. For example, silver particles are 100 times more effective than silver salts as antiseptics. Thus, it is important to prepare nano-sized silver particles or silver alloyed particles to maximize the performance of silver in killing microorganisms. Although some application using silver nanoparticles in colourimetric detection has also been reported recently. Generally, this kind of sensor should be functionalized due to the unstable property for the bare silver nanoparticles[8].

Dispersed silver nanoparticles are yellow in colour, while aggregated ones are dark green[9]. Han and Li reported a kind of silver nanoparticle sensor for colourimetric assays of melamine. The chemical sensor was prepared using *p*-nitroaniline (pNA) functionalized silver nanoparticles. The commercial available *p*-nitroaniline was used both as an electron acceptor and as the stabilizer for silver nanoparticles to form the donor-acceptor interaction between the melamine target and *p*-nitroaniline at the silver nanoparticle interface for direct visualization of melamine[10]. Here, *p*-aminobenzene sulfonic acid (*p*-ABSA) was initially developed as a capping agent in the synthesis of silver nanoparticles and then as a receptor for the new prepared sensor. The new prepared sensor has been successfully used to recognize a target of 5-fluorocytosine with a high selectivity and sensitivity.

EXPERIMENTAL

Silver nitrate (99.99 %) were purchased from China National Pharmaceutical Group Corporation. 5-Fluorocytosine, cytosine, thymine, guanoaine, hypoxanthine, 5-chlorouracil, 2, 6-dichloropurine, 2',3',5'-*tri-o*-acetyluridine with a purity of 99 % was donated from Xinxiang TuoXin Biochem. Tech. Inc. (Henan, China). Sodium borohydride, *p*-aminobenzene sulfonic acid and other reagents were purchased from Beijing or Shanghai Chemical Reagent Limited Company, China and used without further purification. All chemicals used are of analytical grade or of the highest purity available. UV-VIS

absorption spectra were acquired on a UV-2100 spectrometer (Beijing Beifen-Ruili Analytical Instrument Co. Ltd.). IR spectra were measured with a NEXUS FT-IR spectrometer (Thermo Nicolet Co.).

Preparation of the *p*-aminobenzene sulfonic acid-modified silver nanoparticles: Silver nanoparticles were prepared according to the previous reports by reduction of silver nitrate salts with sodium borohydride, but the method was modified using *p*-aminobenzenesulfonic acid instead of *p*-nitroaniline in the preparation process[10]. The synthesis process can be abbreviated as follows: An ethanolic solution of CS_2 (1 mL, 1 mM) was added dropwise to a solution of *p*-aminobenzene sulfonic acid (1 mL, 1 mM) and sonicated for 5 min. 9 mg of $NaBH_4$ was added to 100 mL of 0.1 mM $AgNO_3$ solution with stirring for 15 min to form metallic silver nuclei. And then, the above mentioned CS_2-*p*-ABSA reaction mixture was added rapidly and continuous stirred for 2 h at room temperature. The bright yellow *p*-ABSA-modified silver nanoparticles were finally obtained. The synthesized Ag NPs were characterized by IR, which exhibits the characteristic features of the benzene ring moiety at 3218, 1035 and 1502 cm^{-1} and N-H moiety at 787 and 3430 cm^{-1}. Compared with the IR spectrum of Ag NPs, new bands are observed for *p*-ABSA-modified Ag NPs: (C-S) 1009 cm^{-1} and (CS-NH) 1229 cm^{-1}, indicating a successful formation of the dithiocarbamate-*p*-ABSA ligand. The result indicated that *p*-ABSA was successfully modified on the surface of silver nanoparticles *via* the carbodithioate (-CS_2) linkage.

RESULTS AND DISCUSSION

Detection mechanism and selectivity for the chemical sensor: The charge transfer interactions between an electron donor and electron acceptor has been successfully applied to many interesting studies[11-14]. For example, the electron donor-acceptor interaction between ligand and target analyte induced the colour change of nanoparticles has been reported to detect 2,4,6-trinitrotoluene, thiol-containing amino acids and peptides. The electron-rich surface of the target acting as an electron donor can interact with electron acceptor ligands such as *p*-nitroaniline, through electron donor-acceptor interaction. By utilizing *p*-aminobenzene sulfonic acid as a stabilizer, *p*-aminobenzene sulfonic acid-modified silver nanoparticles were prepared by a conventional sodium borohydride reduction method. The ligand *p*-aminobenzene sulfonic acid was attached directly to silver nanoparticles surface *via* zero-length covalent coupling. This was accomplished by using carbon disulfide (CS_2) as a linker. The CS_2 reacted with *p*-ABSA to form dithiocarbamates (DTCs). The resulting DTCs are strongly bound to the surface of silver nanoparticles, leading to a secure coating of nanoparticles. Silver nanoparticles, a good electron affinity, are known to bind well with ligands containing lone pair electron *via* the coordination bond.

A great challenge of visual detection using it is to develop an effective method for the special target molecular with high sensitivity and selectivity[15,16]. In order to examine the specific recognition for the target using the modified silver nanoparticles, control experiments in Microlon ELISA Plates 96 were carried out using eight compounds including 1) 5-fluorocytosine, 2) cytosine, 3) thymine, 4) guanoaine, 5) hypoxanthine, 6) 5-chlorouracil, 7) 2,6-dichloropurine and 8) 2',3',5'-*tri-o*-acetyluridine. The molecular structure for eight compounds are shown in Fig. 1. In the comparative experiment, no colour changes were observed except 5-fluorocytosine in the selective concentration lower than 0.02 mg/L (Fig. 2). Furthermore, the comparative experiments were also carried out using 0.15 mM modified silver nanoparticles in the presence of 0.01 mg/L different analytes with a total volume of 2 mL, respectively. The colour change of the silver nanoparticles solution in the presence of 5-fluorocytosine was monitored by UV-VIS spectroscopy. The absorption spectrum exhibited a large change after the addition of 5-fluorocytosine: a new and strong absorbance peak appeared at 645 nm from 390 nm, which was ascribed to the absorbance of the aggregated silver nanoparticles. Such a change in the spectrum and the solution colour could be well understood by the 5-fluorocytosine induced aggregation of the *p*-ABSA-stabilized silver nanoparticles through electron D-A interactions between 5-fluorocytosine and *p*-ABSA, where 5-fluorocytosine acts as electron donor and *p*-ABSA acts as electron acceptor. It was attributed that the amino group of 5-fluorocytosine and stereo-hindrance effect led to the different interaction for different analytes. Moreover, only 5-fluorocytosine shows a significantly higher absorption ratio (A_{645}/A_{390}) more than 0.2 and a green colour (Fig. 3). Other analytes with similar structure

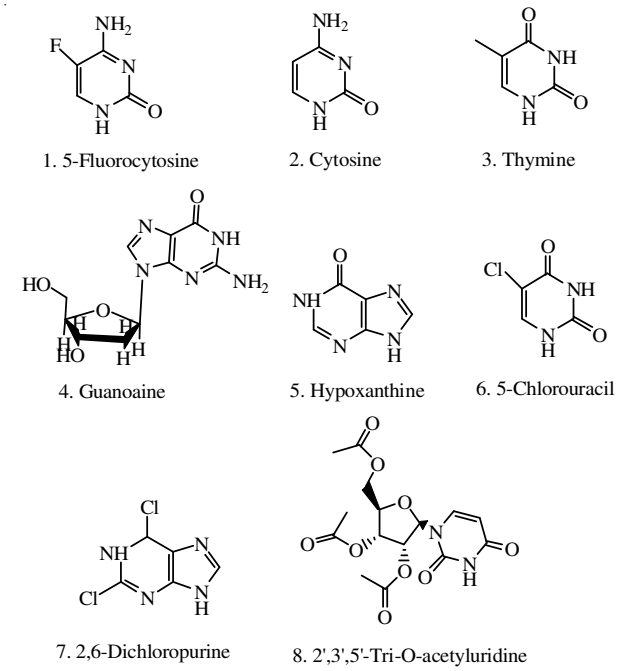


Fig. 1. Molecular structures of eight model compounds

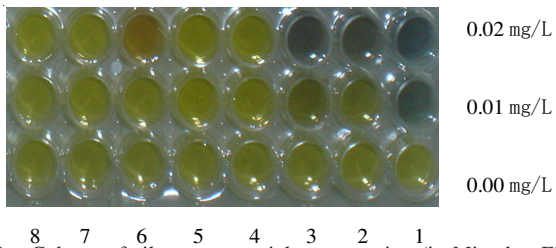


Fig. 2. Colours of silver nanoparticles suspension (in Microlon ELISA Plates) after the addition of eight analysts with different concentrations, respectively

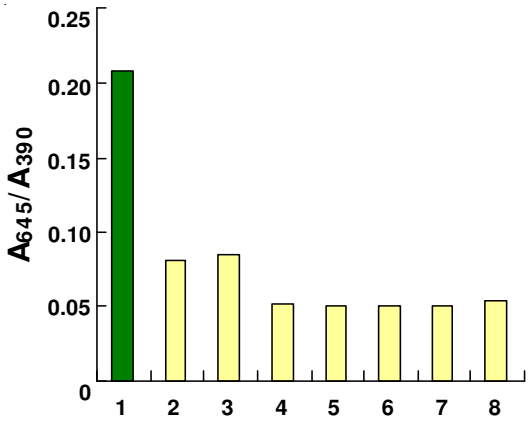
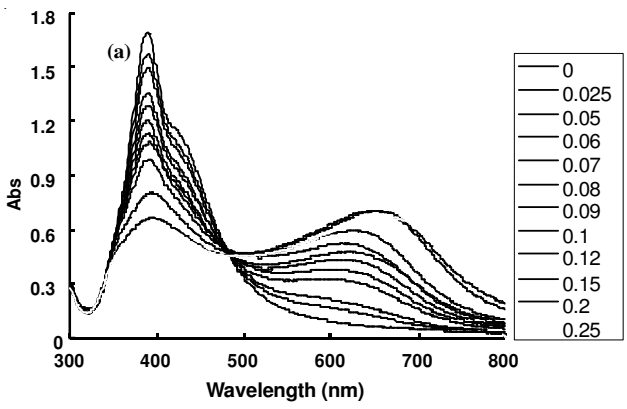


Fig. 3. Absorption ratio and colours of silver nanoparticles suspension after the addition of each analyst with a concentration of 0.01 mg/L, respectively

can not interfere in the detection of 5-fluorocytosine with an original yellow colour and lower absorption ratio less than 0.1. All mentioned above indicates that the modified silver nanoparticles have a specific response towards 5-fluorocytosine.

Standard curve and stability: In order to quantitatively detect the model analyte using prepared silver nanoparticles colourimetric sensor, UV-VIS absorption spectra of silver nanoparticles in the absence and presence of different concentrations of 5-fluorocytosine were recorded with a wavelength in the range of 300-800 nm. With the addition of different concentration of 5-fluorocytosine from 0 to 0.25 mg/L, the colours of silver nanoparticles changed from yellow to green (> 0.06 mg/L) progressively. The relative spectra and the absorption ratio verse the target concentration was also plotted in Figs. 4a and 4b, respectively. It exhibited a linear correlation ($y = 5.8086x - 0.1162$, $R^2 = 0.9823$) between the absorption ratio and 5-fluorocytosine concentration in the range of 0.025 to 0.25 mg/L. This colour change from red to green indicates that more and more silver nanoparticles were consumed to form more and more aggregates.

The time-dependent absorbance changes upon the reaction of the *p*-ABSA-modified silver nanoparticles with 5-fluorocytosine are plotted in Fig. 5. The absorbance ratio A_{645}/A^{390} is intensified as the reaction time is prolonged and it levels off to a saturation value, corresponding to the equilibration of the reaction. It can be seen that the absorption ratio (A_{645}/A_{390}) increased gradually from 1 min to 10 min and kept steady from 10 min to 50 min, demonstrating that the aggregation of silver nanoparticles almost completed within 10 min.



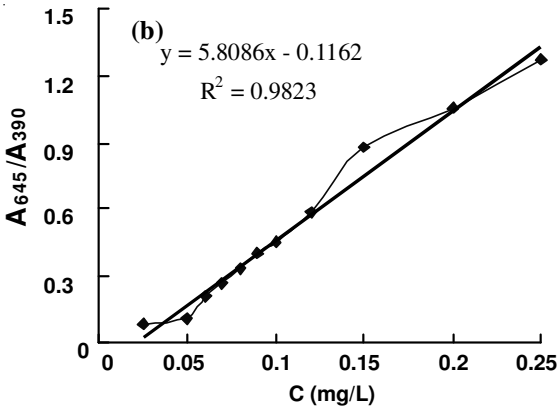


Fig. 4. (a) Evolution of UV-VIS absorbance spectra of silver nanoparticles suspension with the concentration of 5-fluorocytosine from 0 to 0.25 mg/L; (b) The corresponding absorbance ratio (A_{645}/A_{390}) *versus* the concentration of 5-fluorocytosine

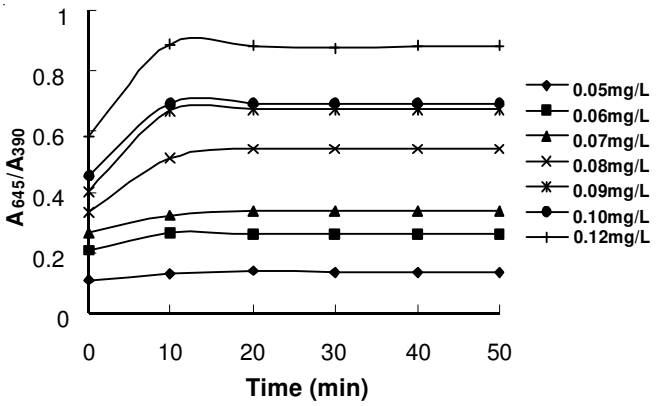


Fig. 5. Time-dependent absorbance changes upon the interaction between modified silver nanoparticles and 5-fluorocytosine target in the concentration range of 0.05-0.12 mg/L

Effect of pH and salt concentration: As pointed out earlier, the formation of silver nanoparticles influences the colourimetric signal, which is associated with the interaction between the target and the silver nanoparticles. Therefore it is speculated that the sensitivity and sensing range of the colorimetric signal are related to the resistance of silver nanoparticles to aggregate, so it is possible to adjust the detection sensitivity and dynamic range to a desired concentration range by the addition of ionic solution in our prepared modified silver nanoparticles colloids. As shown in Fig. 6, obvious changes of the absorption ratio (A_{645}/A_{390}) are determined for the reaction mixture of silver nanoparticles and target of 5-fluorocytosine with NaCl at 5 mM and 10 mM, respectively. Here, 5-fluorocytosine in the range of 0-0.15 mg/L was added into the modified silver nanoparticles colloids, respectively. Generally, visually colour changes from yellow to green would occur with a mutation point (A_{645}/A_{390}) of being about at 0.2. For example, a significant colour change (from yellow to dark green) occurred for *p*-ABSA-modified silver nanoparticles with 10 mM Na_2SO_4 in the absence of target compound of 5-fluorocytosine. Moreover, a significant colour change (from yellow to dark green) occurred for *p*-ABSA-modified silver nanoparticles with 5 mM Na_2SO_4 when the target concentration of 5-fluorocytosine is larger than 0.03 mg/L. The measurements of extinct ratios further demonstrate that Na_2SO_4 is capable of improving the sensitivity of the proposed method.

Conversely, pH dependence was also investigated for the identical process in the range of 3.0-11. Low pH generally resulted in high sensitivity for visualization, for instance, a significant colour change could be observed from yellow to white for modified silver nanoparticles collides in pH 3-4, which was attributed the aggregates formed the precipitation. The relation of absorbance ratio and pH was given in Fig. 7. When the target concentration was lower than 0.1 mg/L, the high sensitivity could be carried out by adjusting pH in the range of 9-10. Moreover, the obvious changes of sensitivity could be achieved in the pH range of 7-8 with a target concentration more than 0.1 mg/L. The adjust ability in sensitivity and dynamic range was very important toward practical applications. For example, to determine whether a sample contains 5-fluorocytosine above a certain safety level, *p*-ABSA-modified silver nanoparticles can be preconditioned so that a significant colour change occurs around the safety level and is added to the sample as a prescreen.

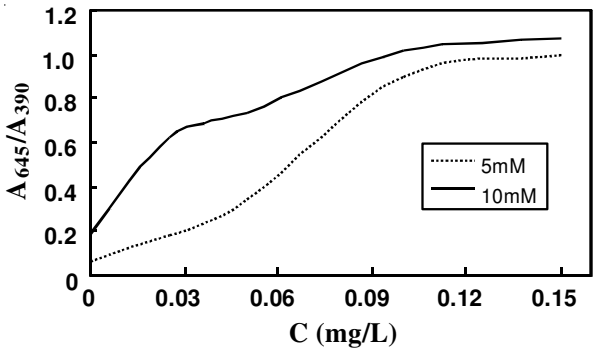


Fig. 6. Na_2SO_4-dependent colourimetric analysis of 5-fluorocytosine using modified silver nanoparticles

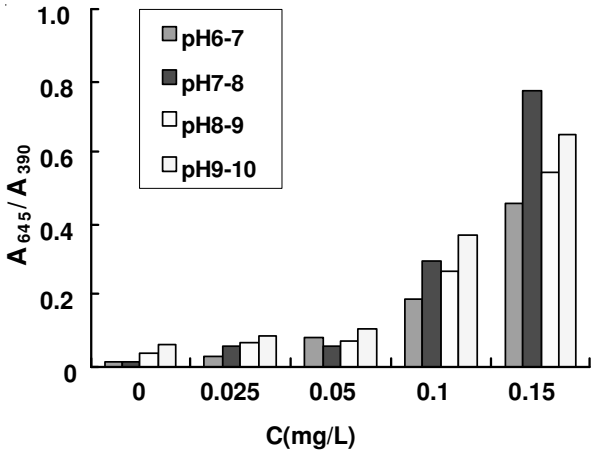


Fig. 7. pH-dependent colourimetric analysis of 5-fluorocytosine using modified silver nanoparticles

Conclusion

In summary, a sensitive, selective and simple colourimetric sensor was prepared to detect 5-fluorocytosine without the aid of any advanced and expensive instrument. The modified-silver nanoparticles probe has a good sensitivity for detection of target, the absorption ratio linearly increased with the increasing concentration of 5-fluorocytosine. With the help of UV-VIS spectrometer, the detection limit of the proposed method is very low. Thus, this colourimetric assay is promising for on-site and real-time detecting 5-fluorocytosine in some actual products.

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