Preparation of Silver Nanoparticle and Its Application to the Determination of ct-DNA

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Abstract: A new method to prepare silver nanoparticles was reported in this paper, which based on aqueous-gaseous phase reaction of silver nitrate solution and ammonia gas. The proposed method is effective, rapid, and convenient. Transmission electron microscope (TEM) and X-ray diffraction (XRD) were used to characterize the structure of the particles, respectively. It was found that the average diameter of the particles was about 10 nm and the shape was spherical. And the strong fluorescence signal of the silver nanoparticles solution can be quenched after the adding of the calf thymus DNA (ct-DNA) solution. The quenched fluorescence intensity was linear with the concentration of ct-DNA in the range of 0.5 to 5.0 × 10^4 ng mL^{-1} with a detection limit of 0.3 ng mL^{-1}.

Keywords: silver, nanoparticle, fluorescence, calf thymus DNA (ct-DNA)

1. Introduction

In recent years, noble metal nanoparticles have been extensively studied and various approaches have been employed for the preparation of metal nanoparticles [1-6]. Among the noble metal nanoparticles, silver nanoparticles have attracted more attention [7-13] for their advantage on various studies such as photosensitive components [14], catalysts [15-17], and surface-enhanced Raman spectroscopy [18-19]. In this paper, we prepared a kind of silver nanoparticles by the reaction of silver nitrate solution and
ammonia gas. The products were characterized by powder X-ray diffraction (XRD), transmission electron microscope (TEM) and fluorescence spectrophotometer, respectively. The results showed that the particles were about 10 nm average diameter and spherical shape. And the strong fluorescence signal would emit when exciting the solution of the nanoparticles by the light with appropriate wavelength. To our best knowledge, quantitative analysis of DNA is very important for medical and biological research [20-23]. A number of methods for the determination of DNA have been established. However, many of them require special reaction conditions. Here, we proposed a new spectrofluorimetric method to determine ct-DNA rapidly by fluorescence characteristic of the silver nanoparticles. In our work, we found that strong fluorescence signal generated from the silver nanoparticles solution can be quenched when a ct-DNA solution was added in. Based on these observations, a new spectrofluorimetric method was proposed for the determination of ct-DNA, with a wide linear range of 0.5 to $5.0 \times 10^4$ ng mL$^{-1}$ and the detection limit of 0.3 ng mL$^{-1}$.

2. Experimental

Fluorescence spectra were recorded on a F-4500 fluorescence spectrophotometer (Hitachi, Japan). Morphological measurement of the silver nanoparticles was performed by a JEM-100CX transmission electron microscopy (JEOL, Japan). XRD pattern of the silver nanoparticles was obtained by a DX-1000 X-ray powder diffractometer (Dandong, China). UV absorption spectrum was recorded on a UV-1100 spectrophotometer (Shanghai, China).

All reagents used were of analytical grade without any further purification. Calf thymus DNA (ct-DNA) was purchased from Sigma (U.S.). Silver nitrate and ammonia solution were purchased from Chengdu Chemical Reagent Corporation (Chengdu, China). Double distilled water was used throughout the experiment.

The apparatus used in the experiment is shown in Fig. 1. Added 50 mL silver nitrate solution ($1.0 \times 10^{-3}$ mol L$^{-1}$) into a 500 mL flask (A), which was placed into a constant temperature water bath on a magnetic stirrer. Then 50 mL ammonia solution (1.0 mol L$^{-1}$) was added into another 500 mL flask (D). Flasks A and D were connected with glass tubes and short pieces of rubber tubes, through which the ammonia gas in flask D volatilized and diffused slowly into the flask A and reacted with the silver nitrate solution. In all stirring procedures of preparing silver nanoparticles, the vessel was exposed to the light of a daylight lamp (40 W) at a distance of 100 cm. The whole experiment lasted 54 hours. The detailed procedure of preparing silver nanoparticles contained five steps: (1) keep the reaction 11 hours under stirring (~39 °C water bath); (2) keep the reaction for 13 hours without stirring and heating; (3) keep the reaction for 10 hours (conditions are same as step 1); (4) repeat the step 2; (5) keep the reaction for 7 hours (conditions are same as step 1). This synthetic method, which proposed in this work, was based on the following reactions:

$$\text{Ag}^+ + \text{OH}^- \rightarrow \text{AgOH}$$

$$\text{AgOH} \rightarrow \text{Ag}_2\text{O}$$

$$\text{Ag}_2\text{O} \rightarrow \text{Ag}$$
3. Results and Discussion

3.1. Optimization of General Procedure

3.1.1. Effect of Reactant Concentrations

In the course of preparing silver nanoparticles, the concentration of silver nitrate solution and ammonia solution played an important role. The different experimental phenomena depended on the preparation conditions were listed in Table 1. Hence, their concentrations should be carefully optimized to ensure preparation success. Under the appropriate condition, the solution of nanoparticles should have strong fluorescence without any precipitation, which can not be observed when the concentrations of silver nitrate and ammonia solutions were too low or too high. Finally, the optimum concentrations of silver nitrate and ammonia solutions were set to $1.0 \times 10^{-3}$ mol L$^{-1}$ and 1.0 mol L$^{-1}$, respectively. Under the optimum conditions, the produced particles exhibited highest fluorescence intensity and no precipitation occurred as listed in Table 1.

| No. | Reagents       | Concentration (mol L$^{-1}$) | Phenomena | Precipitation | Fluorescence |
|-----|----------------|------------------------------|-----------|---------------|--------------|
| 1   | AgNO$_3$       | $1.0 \times 10^{-5}$         | No        | No            |              |
|     | NH$_3$.H$_2$O  | $1.0 \times 10^{-5}$         |           |               |              |
| 2   | AgNO$_3$       | $1.0 \times 10^{-5}$         | No        | No            |              |
|     | NH$_3$.H$_2$O  | 1.0                          |           |               |              |
| 3   | AgNO$_3$       | $1.0 \times 10^{-4}$         | No        | No            |              |
|     | NH$_3$.H$_2$O  | 1.0                          |           |               |              |
| 4   | AgNO$_3$       | $1.0 \times 10^{-3}$         | No        | Yes           |              |
|     | NH$_3$.H$_2$O  | 1.0                          |           |               |              |
| 5   | AgNO$_3$       | $1.0 \times 10^{-2}$         | Yes       | Yes           |              |
|     | NH$_3$.H$_2$O  | 1.0                          |           |               |              |
| 6   | AgNO$_3$       | 1.0                          | Yes       | Yes           |              |
|     | NH$_3$.H$_2$O  | 1.0                          |           |               |              |
3.1.2. Effect of Reaction Time

Due to a significant effect of the reaction time of the preparation, different reaction time experiments were carried out. From Table 2, it is found that no precipitation and fluorescence were observed when the reaction time was less than 20 hours. The fluorescence began to appear after 20 hours’ reaction and increased with the reaction time. However, the fluorescence intensity of nanoparticles was low between 20 and 40 hours. After reacting 60 hours, obvious precipitation occurred. Therefore, the optimum reaction time for the preparation was selected to be 54 hours.

Table 2. Effect of reaction time.

| No. | Reaction time (hours) | Precipitation | Phenomena | Fluorescence |
|-----|-----------------------|---------------|-----------|--------------|
| 1   | 2                     | No            | No        |              |
| 2   | 6                     | No            | No        |              |
| 3   | 10                    | No            | No        |              |
| 4   | 20                    | No            | No        |              |
| 5   | 30                    | No            | Weak      |              |
| 6   | 40                    | No            | Weak      |              |
| 7   | 53                    | No            | Strong    |              |
| 8   | 54                    | No            | Strong    |              |
| 9   | 55                    | No            | Strong    |              |
| 10  | 60                    | Yes           | Strong    |              |

3.2. Fluorescence Spectral Characteristics of the Silver Nanoparticles

Fig. 2 shows the excitation and emission spectra of the silver nanoparticles. Two excitation peaks appeared at the wavelength of 239 and 314 nm respectively by emitting at 384 nm. In aqueous medium, the silver nanoparticles had a peak at 384 nm (curve C) with an excitation at 239 nm. Another emission peak (curve B) of the nanoparticles also occurred at 384 nm without any shift by changing excitation wavelength from 239 nm to 314 nm, which indicated that curve B and C were not scattering peaks but fluorescence peaks. The shape and intensity of the two peaks in excitation spectrum A are well in agreement with the curve B and C, respectively. In addition, no significant change was observed in the repetitive experiments within one month, which exhibited excellent stability of the nanoparticles.

Fluorescence tests of regents used in the experiment and by-products were carried out in order to confirm the fluorescence emitted from the nanoparticles. In any spectrogram of Fig. 3, shape and location of the peak excited at 239 nm (black line) are all different from the peak excited at 314 nm (red line), which indicates all these peaks are scattering peaks but not fluorescence peaks. We also investigated spectra of possible by-products, Ag(NH₃)₂OH and Ag₂CO₃, as shown in Fig. 4. For the same reason, the peaks are not fluorescence peaks either. By thorough analysis, we excluded the possibility of fluorescence emitted from regents and possible by-products.
**Figure 2.** Fluorescence spectra of silver nanoparticles (A. excitation spectra emitted at 384 nm; B. emission spectra excited at 314 nm; C. emission spectra excited at 239 nm).

**Figure 3.** Scattering spectra of reagents (Red lines represent peaks excited at 314 nm, and black lines represent peaks excited at 239 nm. A. H$_2$O; B. AgNO$_3$; C. NH$_3$.H$_2$O).

**Figure 4.** Scattering spectra of Ag(NH$_3$)$_2$OH and Ag$_2$CO$_3$ (Red lines represent peaks excited at 314 nm, and black lines represent peaks excited at 239 nm).
3.3. **TEM, ED and XRD Analysis**

The size and morphology of particles were investigated by TEM (transmission electron microscope) and ED (electron diffraction) (Fig. 5). The TEM image indicates that the silver particles are about 10 nm average diameter and spherical shape. And ED image reveals that the silver nanoparticles are polycrystalline structure.

**Figure 5.** TEM image and the corresponding ED pattern (inset) of silver nanoparticles sample.

The typical powder XRD pattern of the prepared nanoparticles is shown in Fig. 6. The data shows diffraction peaks at $2\theta = 38.2^\circ, 44.4^\circ, 64.6^\circ, 77.5^\circ,$ and $81.7^\circ,$ which can be indexed to (111), (200), (220), (311), and (222) planes of pure silver (PDF № 04-0783). It confirmed that the main composition of the nanoparticles was silver. It is also found that there are some little peaks which match with the standard XRD data of $\text{Ag}_2\text{CO}_3$ (PDF № 26-0339). The silver carbonate formation may be attributed to carbon dioxide in atmosphere. Therefore the product should be preserved in an airproof condition.

**Figure 6.** XRD pattern of the silver nanoparticles.

3.4. **Determination of ct-DNA**

As shown in Fig. 7, the effect of the concentration of the silver nanoparticles has also been investigated. At the beginning, with increasing the concentration of the nanoparticles solution, the
fluorescence intensity of the system increased. However, the intensity increased very slightly after the concentration of the nanoparticles reached $5.0 \times 10^{-4}$ mol L$^{-1}$, and it dropped slowly when the concentration of nanoparticles reached $8.0 \times 10^{-4}$ mol L$^{-1}$.

![Graph of fluorescence intensity vs. silver nanoparticles concentration](image)

**Figure 7.** Effect of the concentration of the silver nanoparticles on the relative fluorescence intensity.

When the concentration of the nanoparticles solution was low, the fluorescence intensity decreased which resulted in narrow linear range of detection of ct-DNA. However, sensitivity can be improved because of obvious quenching response to adding little ct-DNA. Increasing the concentration of the nanoparticles solution to a high value, the enhanced fluorescence led to a wide linear range of detection with sensitivity reducing. At last, in order to acquire both high sensitivity, and wide linear range, the nanoparticles concentration of $5.0 \times 10^{-4}$ mol L$^{-1}$ is recommended.

Under the optimum conditions defined, spectra of fluorescence quenching of the nanoparticles solutions containing different concentrations of ct-DNA are shown in Fig. 8. The strong fluorescence of silver nanoparticles can be quenched in the presence of ct-DNA (shown in Fig. 8 b-i). As can be seen in Fig. 9, the linear detection range from 0.5 to $5.0 \times 10^{4}$ ng mL$^{-1}$ was achieved. The calibration equation of the best fitted line was $\lg[(F_0/F-1)/0.05]=0.2319\lg(C/0.05)-0.2777$, with a correlation coefficient of 0.9953 ($n = 11$, $RSD = 3.6 \%$), and a detection limit of 0.3 ng mL$^{-1}$. $F_0$ and $F$ were the fluorescence intensities before and after the addition of ct-DNA, respectively, and $C_i$ was the concentration of ct-DNA.

3.5. Energy Transfer between the Silver Nanoparticles and ct-DNA

The overlap of the UV absorption spectrum of ct-DNA with the fluorescence emission spectrum of the nanoparticles is shown in Fig. 10. It is found that there is an intersection of UV absorption spectrum and fluorescence emission spectrum. Thus the energy transfer from nanoparticles to ct-DNA can be happened, which can lead to the quenching of fluorescence. The more clear mechanism of the fluorescence quenching in this experiment still requires further study, which is on progress in our lab.
Figure 8. Fluorescence quenching of the nanoparticles solutions containing different concentrations of ct-DNA. $\lambda_{em} = 384$ nm. The nanoparticles concentration (mol L$^{-1}$): $5.0 \times 10^{-4}$. ct-DNA concentration (ng mL$^{-1}$): a. 0; b. 0.5; c. 5.0; d. $5.0 \times 10^3$; e. $2.5 \times 10^3$; f. $5.0 \times 10^3$; g. $5.0 \times 10^3$; h. $2.5 \times 10^4$; i. $5.0 \times 10^4$.

Figure 9. Plot of fluorescence of silver nanoparticles solution quenching by ct-DNA (The nanoparticles concentration: $5.0 \times 10^{-4}$ mol L$^{-1}$).

3.6 Interference of the Coexisting Foreign Substances

The influences of foreign coexisting substances such as bovine serum albumin, glucose, amino acid and ions were tested. As listed in Table 3, most of the examined coexisting substances had no remarkable interference on the assay. From the results, we can see that interference of bovine serum albumin, amino acid and glucose were very weak. Among the tested ions, Mg$^{2+}$, Zn$^{2+}$, Fe$^{3+}$, Ca$^{2+}$, SO$_4^{2-}$, Cl$^-$ and I$^-$ can be allowed with relatively higher concentrations, but Cu$^{2+}$ and PO$_4^{3-}$ ions can only be allowed with relatively low concentrations. The allowed concentrations of these interfering substances, however, were still rather higher than that of ct-DNA, which indicated that this method had a high selectivity.
Figure 10. The overlap of the fluorescence spectrum of the silver nanoparticles ($\lambda_{em} = 384$ nm) (A) with the absorption spectrum of ct-DNA (B).

Table 3. Tests for the interference of co-existing substances.

| Co-existing Substance | Co-existing concentration ($\mu$g mL$^{-1}$) | Change in intensity of fluorescence (%) |
|-----------------------|---------------------------------------------|-----------------------------------------|
| Bovine Serum Albumin  | 5.0                                         | +2.1                                    |
| Glucose               | 20.0                                        | +2.0                                    |
| L-Lysine              | 15.0                                        | -3.3                                    |
| L-Tyrosine            | 10.0                                        | -4.0                                    |
| Glutamic acid         | 16.0                                        | +1.5                                    |
| Glycine               | 13.0                                        | +2.1                                    |
| L-Histidine           | 5.0                                         | +1.2                                    |
| $\text{SO}_4^{2-}$    | 5.5                                         | +1.5                                    |
| $\text{PO}_4^{3-}$    | 2.0                                         | +4.0                                    |
| $\text{Cl}^-$         | 6.0                                         | +3.5                                    |
| $\Gamma$              | 5.5                                         | +3.7                                    |
| $\text{Zn}^{2+}$      | 10.0                                        | +1.6                                    |
| $\text{Cu}^{2+}$      | 2.0                                         | +4.1                                    |
| $\text{Mg}^{2+}$      | 15.0                                        | +1.8                                    |
| $\text{Fe}^{3+}$      | 6.0                                         | +3.5                                    |
| $\text{Ca}^{2+}$      | 15.0                                        | +1.9                                    |

Silver nanoparticles, $5.0 \times 10^{-4}$ mol L$^{-1}$ and ct-DNA, 1.0 $\mu$g mL$^{-1}$.

4. Conclusions

In summary, silver nanoparticles were prepared by the gas-solution reaction of silver nitrate solution and ammonia gas. The silver nanoparticles were about 10 nm average diameters and spherical shape. The fluorescence of silver nanoparticles was quenched by adding ct-DNA, which provided a simple and rapid spectrofluorimetric method for the determination of ct-DNA. The linear range of concentration of ct-DNA was $0.5 - 5.0 \times 10^4$ ng mL$^{-1}$ and the limit of detection was 0.3 ng mL$^{-1}$. 
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