Is a Stop Solution Necessary for Metal Nanoparticles Aggregation-based Colorimetric Assays?

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Research Article

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Abstract

We report herein for the first time that a stop solution should be used to terminate the metal nanoparticles aggregation reactions especially salt-induced ones to increase the repeatability of the assays. It was found that hydrophobic surfactants have the ability to slow down the aggregation process of MNPs and can be applied as stop solution for aggregation based colorimetric assays. This trick provides accurate and well-repeatable signals since the aggregation of the nanoparticles is a dynamic process and capturing signal in a predetermined time is difficult.

1. Introduction

Today, metal nanoparticles (MNPs)-based colorimetric sensors have been extensively applied to detect toxic metal ions, small organic molecules, proteins, and oligonucleotides by using the “aggregation strategy” [1–3].

MNPs solutions show unique optical features depending on the level of target induced aggregation that can be tracked on their surface plasmon resonance (SPR) spectra. The aggregation leads to change of the color of AuNPs solution from red to blue/purple and that of AgNPs from vivid yellow to orange-red that are accompanied by SPR peak shifting at higher wavelengths. MNPs color change can also be monitored with a UV-Vis spectrophotometer to determine the amounts of target quantitatively [3].

However, the aggregation of MNPs is a dynamic process which is fast at the beginning and slows down then. At low concentration of the target, the small aggregates with long-term stable are formed and with increasing its concentration big clusters are formed which settles down due to the gravitational force effects [4, 5].

In addition, NaCl is sometimes used for assisting aggregation reactions to adjust the detection sensitivity and dynamic range to a desired concentration range [6, 7]. As well as, unmodified MNPs-based colorimetric biosensors use NaCl at relatively high concentration to distinguish folded and unfolded ssDNA, [1, 8, 9]. Since at high ionic strengths, the time course of the MNPs aggregation is much faster, usually data with large errors is obtained. Therefore, it is so important to define the well-repeatable signal capture time with high discrimination between different concentrations to resolve the problem brought by the dynamic aggregation process of MNPs.

Till now, a paper-based readout has been reported to achieve accurate results of AuNPs aggregation-based colorimetric biosensors [10]. In this method, a spectrophotometer equipped with an integrating sphere accessory must be used and the optimum volume of AuNPs solution dropped on paper must be determined before analysis.

Herein, we report for the first time that hydrophobic surfactants can act as stop solution for MNPs aggregation based colorimetric sensors. The hydrophobic surfactants that have been already reported for stabilizing well-dispersed MNPs can also stabilize the aggregates and stop or decrease the rate of the

chemical induced aggregation reaction. A suitable surface coating could not only keep the small aggregates apart from each other, but could also ensure the charge neutrality and steric stability of aggregates in their media [11].

2. Experimental

2.1. Chemicals and materials

All solutions were prepared with doubly distilled deionized water from Shahid Ghazi Pharmaceutical Company (Tabriz, Iran; www.sgco-infusion.com). Lamotrigine (LTG) of pharmaceutical grade were purchased from Arastoo Pharmaceutical Company (Tehran, Iran; www.arasto.com), complying with United States Pharmacopeia. LTG stock solution was made up in methanol and further dilution was made in methanol to give suitable working standard solutions. All stock solutions were stored at 4 °C and protected from light. All reagents used were of analytical grade and purchased from Merck (Darmstadt, Germany; www.merck-chemicals.com).

2.2. Instruments

UV-vis absorption spectra were recorded using a UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan; www.shimadzu.com) with a 1.0-cm quartz cell at room temperature.

2.3. Synthesis of ASA-modified AgNPs

The AgNPs were prepared by reducing AgNO_3 according to a previously reported method [12]. All pieces experimental glassware used was cleaned with regia solution ($\text{HCl}:\text{HNO}_3$, 3:1) and then rinsed thoroughly with doubly distilled deionized water prior to use. 2.0 mL of 0.01 M AgNO_3 and 2.0 mL of 0.01 M ASA were added into 94 mL of double distilled water and stirred vigorously for 20 min. Subsequently, 8.8 mg of NaBH_4 was quickly added into the mixture solution under vigorous stirring. The color of the solution immediately changed to black and after a few seconds turned yellow. The resulting yellow silver colloidal solution was stirred for 2 h in a dark place at room temperature. The ASA-AgNPs were stored at 4°C in a dark place and used directly in the following experiments.

2.4. General procedure of detection and determination of LTG

To investigate the aggregation behaviour of AgNPs in solution, AgNPs (3 mL) was mixed with LTG solution (0.2 mL, $1 \mu\text{gml}^{-1}$) of various concentrations. The mixture solutions were immediately transferred to a 1.0 cm path length curvet and the UV-vis absorption spectra were collected at predetermined intervals. To study the effect of SDS as the AgNP aggregation inhibitor, 0.2 mL SDS (0.015 M) was added in the solution within definite time.

2.5. Synthesis of AuNPs

AuNPs of 13 nm in diameter were synthesized using the citrate reduction method [10]. 10 mL trisodium citrate solution (38 mM) and 100 mL chloroauric acid solution (1 mM) were used to prepare red AuNP solution.

2.6. General procedure of detection and determination of NaCl

To investigate the aggregation behaviour of AuNPs in the solution, NaCl solution (0.2 mL, 30 mM) was mixed with AuNPs (3 mL). The mixture was immediately transferred to a 1.0 cm path length cuvet and the UV-vis absorption spectra were collected at predetermined intervals. To study the effect of CTAB as the AuNP aggregation inhibitor, 0.2 mL CTAB (0.015 M) was added in the solution within definite time.

3. Results And Discussion

To study the effect of hydrophobic surfactants as the nanoparticle aggregation inhibitors, two model systems, NaCl- AuNPs and LTG- amidosulfonic acid capped AgNPs were selected.

The spectra and color change of AuNPs after addition of NaCl (30 mM) at different times (0 min, 3 min, 30 min and 24 hours) are shown in Fig. 1. The spectra of NaCl-AuNPs solution changed a lot from 3 min to 30 min and became almost a horizontal line in 24 hours. It can be seen that AuNPs solution with 30 mM NaCl changed from purple to blue in 30 min and coagulation induced transparent after 24 hours.

Similarly, the spectra of AgNPs-LTG normal solution changed a lot from 3 min to 30 min and became almost a horizontal line in 24 hours. In addition, the color of AgNPs with $1 \mu\text{gml}^{-1}$ LTG changed from yellow to orange in 30 min and precipitation induced transparent after 24 hours (Fig. 2).

To select the most suitable inhibitor for NaCl-AuNPs and LTG-AgNPs systems, the effect of different hydrophobic surfactants including sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium bromide (CTAB), Triton X-100 and polyethylene glycol 600 were estimated on the proposed systems in a constant concentration (data not shown). By adding a suitable stop solution within definite time, the aggregation reaction slows down and remains constant.

The proposed mechanism was confirmed using UV-Vis spectra of AuNPs-NaCl and AgNPs-LTG solutions which were recorded by adding CTAB and SDS after 3 and 15 min to the above systems respectively. According to Figs. 3 and 4, during the recorded time, the spectra of surfactant-stopped solution remained almost constant. In addition, the color of NaCl-AuNP and LTG-AgNP solutions followed by adding CTAB and SDS remained almost unchanged (Figs. 3 and 4).

4. Conclusion

In conclusion, we have found that the hydrophobic surfactants have the ability to slow down the aggregation process of MNPs and can be applied as stop solution for aggregation based colorimetric

assays. This trick provides more stable signals for sensing purposes and solve the problems that were reported.

5. Declarations

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Conflicts of interest

There are no conflicts to declare.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

AS was in charge of research and drafting. AJ were responsible for leading this work and revising the manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

6. References

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Figures

Figure 1

UV-Vis spectra of AuNPs in the presence of 30 mM NaCl at 0 min, 3 min, 30 min and 24 hours. The inset shows the photographs of corresponding solutions.

Figure 2

UV-Visible spectra of ASA capped AgNPs in the presence of 1 µg ml⁻¹ LTG at 0 min, 3 min, 30 min and 24 hours. The inset shows the photographs of corresponding solutions.

Figure 3

UV-Visible spectra of AuNPs in the presence of 40 mM NaCl at 0 min, 3 min, 30 min and 24 hours by adding stop solution (200 μ l of 0.015 mol.L⁻¹ CTAB) after 3 min. The inset shows the photographs of corresponding solutions.

Figure 4

UV-Visible spectra of ASA capped AgNPs in the presence of 1 µg ml⁻¹ LTG at 0 min, 30 min, 2 hour and 24 hours by adding stop solution (200 ul of 0.015 mol.L⁻¹ SDS) after 3 min. The inset shows the photographs of corresponding solutions.