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Separation of different shape biosynthesized gold nanoparticles via agarose gel electrophoresis

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ABSTRACT

A mixture of nanospheres and nanoplates was synthesized through bioreduction of $HAuCl_4$ with *Cacumen Platycladi* leaf extract. In this work, shape separation of the nanospheres and nanoplates was achieved via agarose gel electrophoresis (AGE). Furthermore, the separation mechanism and the effects of parameters, including the agarose gel concentration, electrophoresis voltage and TBE buffer concentration, on the separation performance were investigated. The optimum conditions were found to be 1.2% gel concentration, 100 V voltage, and 1 × TBE buffer solution. Gold nanoparticles (NPs) prepared via a biological method were compared with those synthesized using a chemical method in the electrophoresis process with different characteristics. The results showed that the particles prepared via the biological method had good stability in a buffered solution without further modification.

1. Introduction

The electrical, optical, and magnetic properties as well as the catalytic efficiency of noble metal nanomaterials depend on the particle size and morphology. The synthesis of metal nanoparticles (NPs) with controlled sizes and morphologies is in the limelight in the field of nanotechnology. However, it is still difficult to synthesize metal NPs with uniform size and morphology due to various factors and complex synthetic systems. Therefore, the separation and purification of polydisperse products are of great significance for the improvement of the monodispersity and further applications of the products.

Biosynthesis using plant extracts is a promising alternative to the chemical method used for the production of Au NPs because the preparation process is environmentally benign [1–6]. However, compared with the chemical method, it is more difficult to obtain metal NPs with uniform size and morphology via biosynthesis [7]. In the chemical reduction, the precursor, reductant and protective agent can be added in a step-by-step manner such that controlled nanoparticle size and morphology are relatively simple to achieve. As the plant extract plays a dual role as both the reductant and the protective agent in the bioreduction process of Au NPs, it is difficult to control the morphology of the biosynthesized metal NPs.

Substantial endeavors have been made to purify nanoparticles based on sizes and shapes after preparation [8]. Among the variety of separation methods, gel electrophoresis has long been used in the field of biotechnology because of their scalability and high resolution [9–13]. Tiselius first applied electrophoresis to separate serum proteins in 1937 [14]. Recently, electrophoretic technology has been used to the separation of charged NPs. Hanauer et al. separated spherical, rod-shaped and triangular Ag and Au NPs modified with polyethylene glycol via gel electrophoresis [15], and different color bands were obtained after gel electrophoresis. G. DanieláLilly separated CdTe NPs via free-flow electrophoresis [16], which were modified with a negatively charged mercapto acid. The separation of different sizes of CdTe NPs was achieved in a buffer at pH = 11 using free-flow electrophoresis. Arnaud et al. reported that water-soluble Ag NPs modified with mercaptosuccinic acid could be purified via isoelectric focusing [17]. After loading the Ag NPs directly into a strip with a pH gradient, the isoelectric focusing stopped after the Ag NPs migrated to the zones with the pH values of their isoelectric points, and therefore, separation of Ag NPs with different sizes was achieved. Xu et al. invented a gel electrophoresis device for continuous separation and recovery of Au NPs, which successfully solved the difficulty of particle recovery after electrophoresis [18].

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Currently, separations performance of NPs are performed via gel electrophoresis [18,19]. Unfortunately, few reports focus on the insight into the separation mechanism and the effects of various factors on the mobility as the mobility involves many parameters and uncertainties with respect to the surfaces of the NPs. The mobilities of the particles in the system are dependent on the strength of the applied electric field, the charge of the particles, and the friction between the particles and gels under a moderate electric field strength at low Reynolds number, which is closely related to the particle morphology. In this paper, we present and verify a reasonable assumption and explore the various factors that influence the particle mobility based on an electrophoretic separation mechanism.

2. Experimental

2.1. Materials

Ethylene diamine tetraacetic acid (EDTA) was supplied by Xilong Chemical Reagent Co. Ltd. (China). Agarose gel was supplied by GENE Co. Ltd. Trishydroxymethylaminomethane (Tris), Chloroauric acid ($HAuCl_4{\cdot}3H_2O$), boric acid, sodium citrate were supplied by Sinopharm Chemical Reagent Co. Ltd. (China). All chemicals and reagents were used without further purification. Sun-dried *Cacumen Platycladi* (CP) leaves were purchased from Xiamen Jiuding Drugstore (China).

2.2. Biosynthesis of Au nanoparticles

The sun-dried CP leaves were milled and screened with a 20-mesh sieve. Then, 1.0 g screened powder was dispersed in 100 mL of deionized water and kept in a water bath shaker at 30 °C. After 2 h, the mixture was filtered to obtain the extract. To biosynthesis Au nanoparticles, CP extract and 1 mmol L^{-1} $HAuCl_4$ were injected into a round-bottom flask at a speed of 100 mL h^{-1} through two separate syringe pumps at 90 °C until the total volume was 30 mL. Then, the solution was vigorously stirred for 2 h to produce the Au NPs.

2.3. Chemical synthesis of Au NPs

6 mL 5.9 mmol L^{-1} $HAuCl_4$, 45 mL deionized water and 4 mL 38.8 mmol L^{-1} sodium citrate were mixed in a 150 mL conical flask and kept in a water bath shaker at 100 °C for 30 min.

2.4. Preparation of TBE buffer

20 × TBE: Approximately 108 g Tris, 55 g borate, and 5.84 g EDTA were dispersed in 100 mL of deionized water; this solution was transferred to a 500-mL volumetric flask and diluted with deionized water to the appropriate volume.

1 × TBE: A total of 40 mL of 20 × TBE was mixed with 760 mL of deionized water.

2.5. Preparation of agarose gel

1.2% agarose gel concentration: Agarose (0.24 g) was mixed with 20 mL of 1 × TBE, which was dissolved by heating in a microwave oven. The gel was cooled to 40–50 °C, poured into molds, held for 0.5 h at room temperature, and slowly pulled from the slot.

2.6. Pretreatment of the sample

The samples concentrations were adjusted to 1 mg mL^{-1} using centrifugation with glycerin added to the samples at a volume equivalent to 10% of the sample.

2.7. Electrophoresis

A sufficient amount of 1 × TBE buffer was injected to cover the agarose gel in the electrophoresis tank. Next, the samples were added to electrophoresis tanks of two sizes tank, i.e. one equal to 20 μL and the other equal to 40–50 μL. The Au NPs were separately treated by electrophoresis for 0.5–3 h at 100 V.

3. Results and discussion

3.1. Effect of the concentration of the agarose gel

A mixture of gold nanospheres and nanoplates were prepared according to the previously described biosynthesis method. The images of the gels at various concentrations of agarose gel for AGE separation of Au NPs in Fig. 1 show that two stripes form after AGE separation. The red stripe had greater mobility than the grey green one. As known from earlier experiments, gold nanospheres appear red in solution under visible light while gold nanoplates appear as a grey green color. It indicates that nanospheres and nanoplates could be separated using the AGE method.

Furthermore, the colored stripes became clearer and the Au NP mobility decreased as the gel concentrations increased in a constant electric field. The relationship between the gel concentration and the Au NPs mobility was studied. To analyze the forces on the NPs during electrophoresis, spherical particles with radius R were subjected to three forces, i.e. an electric force F_e, a liquid friction force F_l, and a gel friction force F_g. Furthermore,

$$F_e = F_l + F_g \tag{1}$$

The electric force on the particles is described by the following equation:

$$F_e = E_q \tag{2}$$

where E is the electric field and q is the electric charge. The liquid friction force F_l was assumed according to the Stokes equation:

$$F_l = 6\pi\eta r\upsilon \tag{3}$$

We assumed that the gel friction force F_g was proportional to the gel concentration c:

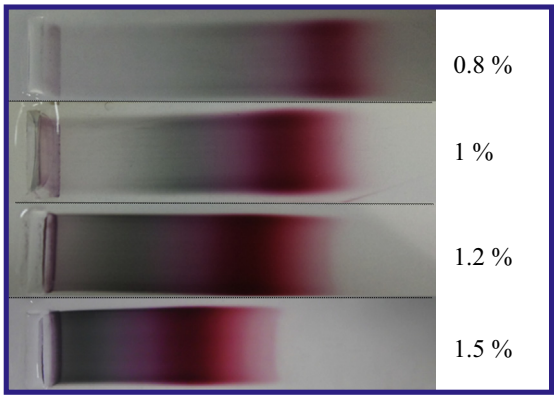

Fig. 1. Image of gels with different concentrations used in the AGE separation of Au NPs (100 V, 1 × TBE buffer solution, 1.5 h).