Research Article

One-Step Synthesis of PEG-Coated Gold Nanoparticles by Rapid Microwave Heating

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Polyethylene Glycol- (PEG-) coated gold nanoparticles (PEG-AuNPs) are synthesized by a one-step route with rapid microwave heating. Homogeneous nucleation of the primary gold particles is enhanced by increasing the applied microwave power during the initial stage of the synthesis, increasing the temperature ramping rate (R_r) and resulting in decreased size and improved uniformity of the synthesized PEG-AuNPs. Using rapid microwave heating, we successfully produce uniform colloidal PEG-AuNPs with an average diameter of 14.3 ± 2.5 nm within a few minutes. By appropriate tuning of the growth parameters, microwave synthesis can produce largely colloidal PEG-AuNPs with high uniformity.

1. Introduction

Colloidal gold nanoparticles (AuNPs) have attracted attention due to the interesting electrical, chemical, and optical properties that have given them the potential for application in a variety of biomedical fields such as sensors [1], disease diagnosis [2–4], therapeutics [5, 6], and others. In particular, AuNPs can be used as image contrast and treatment agents for applications in X-ray radiography and radiotherapy for tumor treatment [7, 8]. The goal of treatment with AuNPs in these fields is for the AuNPs to arrive at the tumor cells after administration into circulatory system and to then enhance the image contrast or treatment effect at the targeted tumor area. In general, two strategies have been developed for the targeting tumors with AuNPs: the passive route and the active targeting route [9, 10]. The passive targeting route depends on the accumulation of AuNPs at tumor areas due to the enhanced permeation and retention (EPR) effect in tumors [11]. The active targeting route uses ligands on the AuNPs surface for recognition by binding to specific cell surface receptors on the tumor. These ligands could be small molecules, peptides, or proteins [11].

In in vivo targeting of AuNPs, it is crucial to bypass the reticuloendothelial system (RES), an essential component of the immune system composed of phagocytic cells. To evade the human body's RES, it is invariably necessary to endow AuNPs with a "stealth" function. Polyethylene Glycol (PEG) has been recommended as a capping material used to provide AuNPs with the requisite stealth against the RES [12]. Several studies have reported that PEG-coated AuNPs (PEG-AuNPs) were successful as image contrast and treatment agents for in vivo targeting [3, 13].

It has been recently emphasized that if PEG-AuNPs are to have practical application in the field as a biomedical agent, they need a synthesis route with mass production capability. Conventional synthesis using chemical reactants in solution is generally carried out through conductive heating with an external heat source. However, this method is inefficient for the uniform transfer of energy into the reactants, resulting in slow reaction rates and a large temperature gradient in the solution that causes nonuniform product formation.

Here, we present a rapid one-step route for preparing highly uniform, nontoxic PEG-AuNPs by microwave heating. We successfully produce uniform colloidal PEG-AuNPs with

(a)

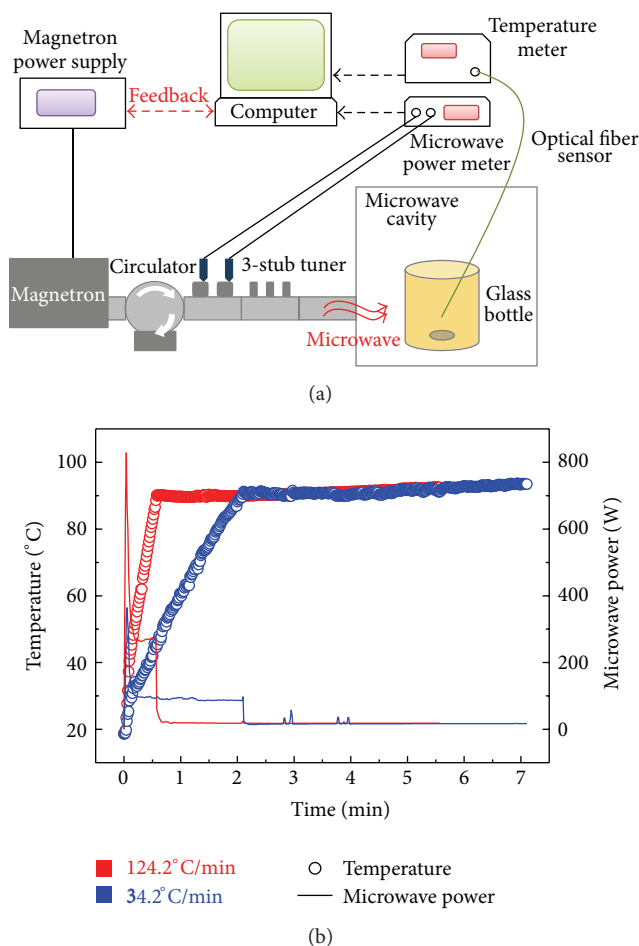124.2°C/min   ○ Temperature
34.2°C/min   — Microwave power

(b)

FIGURE 1: (a) A schematic illustration of microwave heating system. (b) Microwave power (solid line) and solution temperature (open circle) profiles during microwave synthesis of colloidal PEG-AuNPs. The two different temperature ramping rates (R_r) of 124.2°C/min and 34.2°C/min are created by adjusting the applied microwave power during the initial stage (ramping region). After the temperature ramping, the solution temperature is maintained at 90°C using a microwave power of 10 W (retention region).

an average diameter of 14.3 ± 2.5 nm within a few minutes of microwave heating by controlling the applied microwave power during the initial stage of synthesis.

2. Materials and Methods

2.1. Synthesis. A schematic illustration of the microwave heating system (2.45 GHz, 1600 W) used for the entire synthesis of PEG-coated gold nanoparticles (PEG-AuNPs) is shown in Figure 1(a) [14–17]. In this system, the amplitude of the microwave power in the microwave cavity was calculated by measuring the difference between the forward- and backward-propagating microwaves and adjusted using a 3-stub tuner module. The temperature of the reaction solution was measured using a fiber optic sensor that is not affected by microwave radiation. A feedback system between the applied microwave power and the measured temperature allowed for

control of the microwave power to obtain the desired reaction temperature. The solution was placed into a 50 mL glass bottle and stirred with a magnetic stirrer coated with Teflon during the synthesis, and this bottle was placed in the cavity. The growth solution for one-step synthesis was prepared at room temperature by mixing 0.88 mM $HAuCl_4$, 5.0 mM sodium citrate (Na_3Ct), and 0.1 mM PEG (average molecular weight ~20,000/Aldrich) in deionized water (18.2 MΩ · cm). Na_3Ct and PEG were used as a reducing agent and coating material, respectively.

2.2. Measurements. UV-VIS absorption spectra of the synthesized colloidal PEG-AuNPs were analyzed by a Thermo Evolution 300 spectrophotometer. TEM images were taken on a JEM-2100F (JEOL). For TEM analysis, specimens were prepared by placing a drop of colloidal PEG-AuNPs on a carbon-coated copper grid, and evaporating the solvent. The size distribution of the PEG-AuNPs was obtained by measuring the diameter of more than 200 particles. The PEG-AuNPs colloidal solutions were condensed by combining centrifugation and solvent evaporation under reduced pressure. The centrifugation was performed with an Eppendorf 5810R, Germany, centrifuge and an Amicon Ultra-15, Millipore, US centrifugal filter at 4°C. The centrifugation took place at 3200 g for 30 min. The PEG-AuNPs were further condensed by a simple vacuum evaporation system. In order to reveal some of the interactions between the primary gold nanoparticles and their organic ligands, samples of PEG-AuNPs were mixed with IR grade KBr and subjected to FTIR analysis on a Perkin-Elmer FTIR spectrophotometer. All spectra were recorded at a resolution of 4 cm^{-1} over a wave number range of 600–4,000 cm^{-1}.

2.3. Cell Viability Test. EMT-6 cells were cultured in Dulbecco's modified Eagle medium (DMEM/F-12) supplemented with 1% penicillin-streptomycin and 10% heat-inactived fetal bovine serum (Invitrogen, Carlsbad, CA) and were maintained in a humidified incubator with 5% CO_2 and at 37°C; the culture medium was changed every two days. PEG-AuNP colloid was freshly prepared and diluted with Dulbecco's medium. After overnight cell seeding in a multiplate, the EMT-6 cells were cocultured for 24 hours with PEG-AuNPs suspensions with different colloidal concentrations: 0.01, 0.1, or 0.5 mM. Growth medium with no nanoparticles was used for the control specimens. After incubation, some of the cells were harvested and stained by trypan blue reagent (Sigma, St. Louis, MO) to count the number of live cells.

3. Results and Discussion

When microwave radiation is used as a heating source for driving a chemical reaction in water or in an organic solvent with a high dipole moment, rapid volumetric heating can occur, resulting in uniform energy transfer into the solution. In addition, many nonthermal effects of microwave radiation have been previously proposed, including (i) an increase in the probability of collision between two molecules and
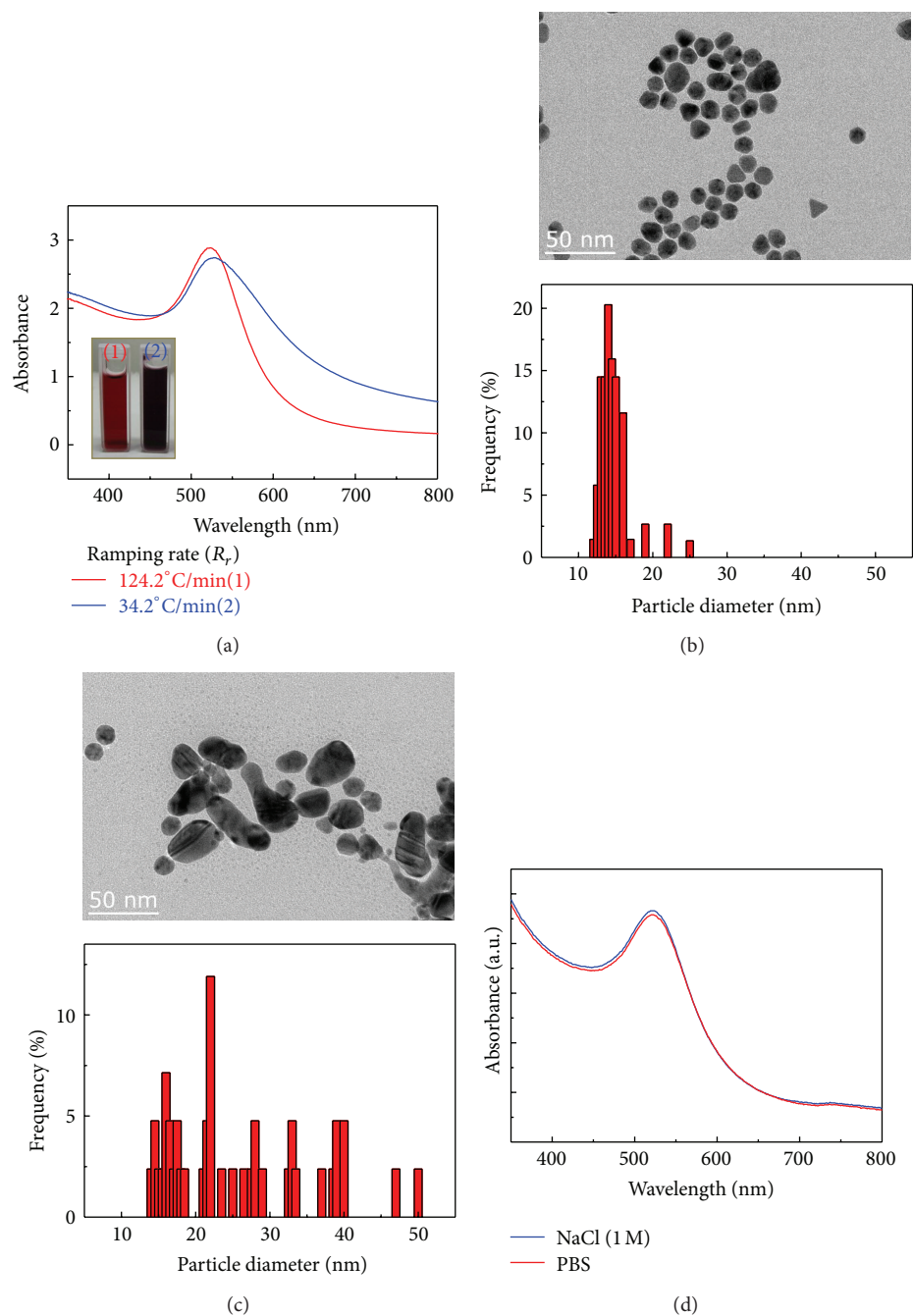
FIGURE 2: (a) UV-vis absorption spectra of the synthesized PEG-AuNPs with respect to R_r. Inset shows color of synthesized PEG-AuNPs. (b-c) TEM images and their corresponding size distributions of the synthesized PEG-AuNPs synthesized at different R_r –124.2°C/min (b) and 34.2°C/min (c). (d) UV-vis spectra of PEG-AuNPs recorded at 24 h with PBS and NaCl.

(ii) a decrease in the activation energy [18] resulting from the direct interaction of the electric fields with specific molecules in the reactants that could enhance reactions. These are the unique characteristics of microwave heating that improve the reaction rates, increase the yields, and increase the uniformity of the products [14–25]. We also note that microwave synthesis is one of the routes by which the need for mass production of nanoparticles can be met.

Shown in Figure 1(b) are the typical profiles of the applied microwave power (solid line) and the corresponding solution temperature (open circle) in the microwave synthesis of colloidal PEG-AuNPs. The profile can be divided into the two temperature regions of "ramping" and then "retention." In the ramping region of Figure 1(b), the solution temperature reaches the desired value of ~90°C using two different ramping rates (R_r) of 124.2°C/min and 34.2°C/min. The R_r

is precisely controlled by adjusting the applied microwave power during the initial stage of synthesis. In the retention region, the solution temperature is maintained at 90°C for 5 min by applying a continuous microwave power of 10 W.

Figure 2(a) shows the UV-vis absorption spectra of PEG-AuNPs synthesized with the two different R_r values and a constant retention time of 5 min in Figure 1(b). As the R_r value increases from 34.2°C/min to 124.2°C/min, the Full Width Half Maximum (FWHM) of the absorbance profile narrows and the maximum peak (λ_{max}) value shows a blue shift from 528 nm to 521 nm, indicating the formation of colloidal PEG-AuNPs with a smaller average size and narrower size distribution. The TEM images and the corresponding size distribution of PEG-AuNPs in Figures 2(b) and 2(c) are clearly well matched with the UV-vis results of Figure 2(a). Both of these results indicate that uniform PEG-AuNPs with an average diameter of 14.3 ± 2.5 nm are produced at the condition of $R_r \sim 124.2$°C/min. Figure 2(d) compares UV-vis absorbance spectra of PEG-AuNPs in presence of 1.0 M NaCl and 1.0 M PBS. No significant shifts in the UV-vis absorbance spectra are observed within at least 24 h, indicating that the resulting PEG-AuNPs are stable at the conditions.

In a previous study, we investigated the formation kinetics of the microwave synthesis of sodium citrate reduced colloidal AuNPs [14]. The uniformity of the synthesized AuNPs was affected significantly by the applied microwave power during the initial stage of synthesis and by the solution pH. The result in Figure 2 can be explained by the influence of the R_r value on the homogeneous nucleation during the initial stage of the PEG-AuNP synthesis. The faster ramp (~124.2°C/min) to the desired temperature of 90°C during the initial stage causes a higher supersaturation ratio of gold atoms in solution. This higher supersaturation results in short nucleation bursts and the production of a large number of small primary AuNPs with high uniformity. With the capping of primary AuNPs with PEG during the growth phase in the temperature retention region, the uniformity of the homogeneous primary AuNPs contributes significantly to the formation of colloidal PEG-AuNPs with a smaller average size and a narrower size distribution. The unmodified PEG molecules used in this process are unlikely to covalently bond to AuNPs; thus PEG molecules are expected to be physisorbed on the gold surfaces. The action of PEG chains is closely related to their affinity to $AuCl^{-4}$ ions and conformation on the primary AuNPs [13, 15, 26, 27]. Conversely, the extended nucleation period due to the slower R_r of 34.2°C/min leads to inhomogeneous nucleation of the primary AuNPs. The AuNPs nucleated during the earlier part of the temperature ramping stage are larger than those nucleated during the later part, resulting in synthesized colloidal PEG-AuNPs with a wide size distribution.

In order to confirm the PEG capping on the surface of AuNPs, we conducted the FTIR analysis of the synthesized nanoparticles. Figure 3 shows the FTIR spectra of typical of bare AuNPs, synthesized PEG-AuNPs, and pure PEG. In contrast to the spectrum of bare AuNPs, IR absorbance peaks are present in the spectrum of PEG-AuNPs, and these peaks are assigned as follows: 2,850–3,000 cm^{-1} (–CH$_2$ stretching),
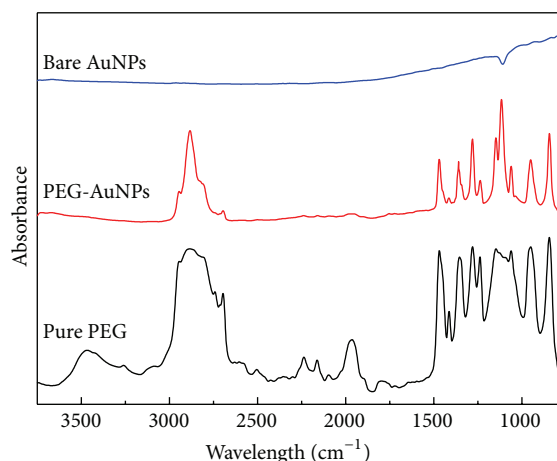

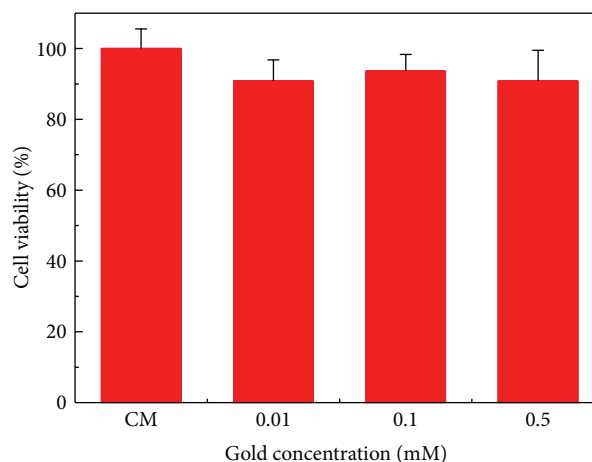FIGURE 3: FTIR spectra of bare AuNPs (blue line), PEG-AuNPs (red line), and Pure PEG (black line).

FIGURE 4: Cell viability of EMT-6 cells cocultured with different concentrations of PEG-AuNPs (CM: control medium).

1,380 cm^{-1} (C–H bending; –CH$_2$ and –CH$_3$), 1,100 cm^{-1} (C–O–C stretching), and 700–900 cm^{-1} (N–H wagging). These peaks are consistent with the spectrum of pure PEG, indicating the adsorption of PEG molecules onto the surface of AuNPs.

Biocompatibility is a critical factor to consider when evaluating the potential of nanoparticles for biomedical purposes, especially in in vivo applications such as image contrast and treatment agents. Biocompatibility is commonly verified by the assessment of the cytotoxicity of nanoparticles. The cytotoxicity of nanoparticles is estimated by measuring the overall dose-dependent toxicity of nanoparticles on cultured cells and determining cell survival after nanoparticle exposure. The cell viability results for the EMT cells exposed to different PEG-AuNPs concentrations are compared to the results for the unexposed control EMT specimen in Figure 4. The cells were treated with 0.01, 0.1, or 0.5 mM concentrations of PEG-AuNPs and cocultured for 24 h, ultimately exhibiting

>90% cell viability. These results show that the synthesized PEG-AuNPs are nontoxic particles.

4. Conclusion

In conclusion, we successfully synthesized highly uniform, nontoxic PEG-coated AuNPs (PEG-AuNPs) with an average diameter of 14.3 ± 2.5 nm in a few minutes by a one-step route with rapid microwave heating. The formation of PEG-AuNPs is affected significantly by the applied microwave power during the initial stage of synthesis. Colloidal PEG-AuNPs with a smaller average size and a narrower size distribution are formed by homogeneous nucleation of primary AuNPs during the initial stage when using a higher temperature ramping rate (R_r). We can anticipate that a large-volume production of PEG-AuNPs with high uniformity can be achieved using one-step microwave synthesis with appropriate control over the applied microwave power.

Acknowledgments

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