

Synthesis and characterization of gold nanocomposites with modified and intact polyamidoamine dendrimers

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Abstract Preparation and characterization of nanocomposites of gold nanoparticles (AuNPs) using 3rd and 4th generation of poly(amidoamine) dendrimers (PAMAM) and modified PAMAM were studied. By utilizing poly-(ethylene glycol) grafted PAMAM G3 (PEG-G3), 9.7 ± 3.1 nm gold particles were obtained; compared to particles of 15.4 ± 7.3 nm size stabilized by unmodified PAMAM G3 dendrimers. In order to decrease the size of the synthesized gold nanoparticles the quaternization of primary amines in the periphery of PAMAM G4 (QG4) dendrimer was suggested. Characterization of the structures by fluorescence spectroscopy, UV-Vis spectrometry and transmission electron microscopy (TEM) revealed that PEG-G3 was better than PAMAM G4 as a templating agent, and that the QG4 dendrimer produced gold nanoparticles with the smallest size and the lowest polydispersity. The average size of gold nanoparticles protected by PAMAM G4 and QG4 were 13.9 ± 4.5 and 6.1 ± 1.6 nm, respectively.

Keywords PAMAM dendrimers · Gold nanoparticles · Nanocomposites · Modified PAMAM

Introduction

Various applications of metal nanoparticles in electronics, magnetic memories, biolabeling and drug delivery have led to further investigation about the synthesis methodologies. Gold nanoparticles of well-defined dimensions, among other metallic nanostructures, have been the focus of attention due to their particular applications and unique structure and properties. For synthesis of nanoparticles, stabilization of metal nanoparticles has been extensively investigated by using a variety of alkanethiols, phosphines, surfactants and polymers [1, 2]. As template and stabilizer, dendrimers are outstanding candidates because of their regular structure and chemical versatility [3–5].

Dendrimers have three basic anatomical features: a core, repetitive branch units, and terminal functional groups [6, 7]. They are produced in an iterative sequence of reaction steps, in which each additional iterations leads to a new generation with doubled molecular weight of the previous generation [8]. Dendrimers were used as a template for the particle formation or a protective agent for the agglomeration of metal particles [9–11]. Many metal particles were prepared in the presence of dendrimers by photoreduction [12] and by the addition of chemical reductants [9]. A variety of dendrimer/metal nanoparticles were prepared in the presence of poly(amidoamine) (PAMAM) dendrimers having surface amino, hydroxyl, or per substituted sugar groups [13–15]. Recently, Hedden and his collaborators [16] reported modifying PAMAM G4 dendrimers with poly(ethylene glycol) (PEG) by grafting PEG onto PAMAM G4 end groups for preparing metal nanoparticles. They detailed

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the desirable effect of improving solubility and miscibility of resulted nanocomposites with solvents by this modification. From a practical standpoint, the improvement in physical properties could facilitate the development and processing of nanocomposites of well-defined dimensions.

Another alternative for preparing diminutive nanoparticles is to employ partially quaternized dendrimers as an efficient template. The permanent positive charges at the dendrimer surface prevent metal ion induced interdendrimer agglomeration.

In this study, PAMAM dendrimers G3, G4, PEG-G3 and quaternized PAMAM G4 (QG4) were exploited as stabilizers for synthesizing gold nanoparticles which have narrow size distribution. Stabilizing ability of these dendrimers was compared to each other and the effect of different parameters on the formation of AuNPs was investigated.

Experimental

Material and methods

All analytical grade chemical reagents and distilled water were used for preparing all aqueous solutions. Hydrogen tetrachloroaurate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), sodium borohydride (NaBH_4) and sodium hydroxide were obtained from Merck (www.Merck.org). The third and fourth generation of PAMAM dendrimers (G3&G4) having amino end groups, as well as PEG-G3 and partially quaternized fourth-generation PAMAM (QG4) were synthesized according to the previously reported methods [17].

Nanocomposite synthesis

Dendrimer-stabilized AuNPs were prepared via two consecutive stages. First, the complex between internal and surface amines of the dendrimer and the gold anions was formed, then the complexed ions were chemically reduced by a reducing agent (NaBH_4) [18–21]. In details 50 μL of 100 μM dendrimer was added to 5 mL of water while vigorously stirred. Aqueous solution of 5 mM HAuCl_4 at defined stoichiometry ratios was added to the above solution. For the complete formation of gold-dendrimer

ionic complexes, the solution was vigorously stirred for further 30 min followed by fast addition a 10 fold molar excess of sodium borohydride in basic aqueous solution (25 mM sodium borohydride in 0.3 M NaOH). The light yellow solution of dendrimer/ HAuCl_4 immediately turned to dark brown, indicating the formation of colloidal gold. The reduction reaction was allowed to proceed for 30 min prior to analysis. In the absence of the dendrimer, the brown solution of AuNPs immediately changed to blue solution of precipitates. The stable brown solution of colloidal gold was indicative of the metal colloid stabilized by the dendrimer.

Characterization and instrumentation

Complexation between aqueous metal ions and dendrimers was monitored by a UV-Vis absorption spectrophotometer (JASCO, Japan, www.jascoint.co.jp) at room temperature, in the range of 190 to 700 nm, using 1 cm long quartz cuvette. The excitation and emission spectra of all dendrimer samples and dendrimer nanocomposites (DNC) were measured using a spectrofluorimeter (Perkin Elmer, USA, www.perkinelmer.com). Transmission electron micrographs were obtained using a transmission electron microscope (Zeiss CEM 902A, Germany, www.smt.zeiss.com/nts) operating at 100 KV. Samples were prepared by placing a drop of DNC solution on a carbon-coated Cu TEM grid, allowing the solvent to evaporate in the air. Zeta potential of the DNC was measured by a Malvern Nano-sizer, ZS series (Malvern Instruments, UK, www.malvern.com).

Results and discussion

Formation of nanoparticles

The stoichiometries used to prepare DNC are listed in Table 1. Figure 1(a) shows the UV-Vis spectra of aqueous solutions containing dendrimer/ AuCl_4^- complexes before reduction with NaBH_4 . The dendrimer solutions in the absence of AuNPs showed only a strong absorption band at 220 nm. However, at the pH of the experiments (about 7.5) and in the presence of HAuCl_4 molecules, the protonated

Table 1 Preparation parameters and zeta potentials of DNCs

DNC	Mol of AuCl_4^-	AuCl_4^- (mM)	Mol of dendrimer	Au: NH_2 group molar ratio	Zeta potential (mV)
G3(Au)	1.59e-7	0.0319	4.99e-9	1:1	23.7
PEG-G3(Au)	1.59e-7	0.0319	4.99e-9	1:1	2.51
G4(Au)	2.75e-7	0.055	4.99e-9	0.85:1	42
QG4(Au)	2.75e-7	0.055	4.99e-9	0.85:1	46.9

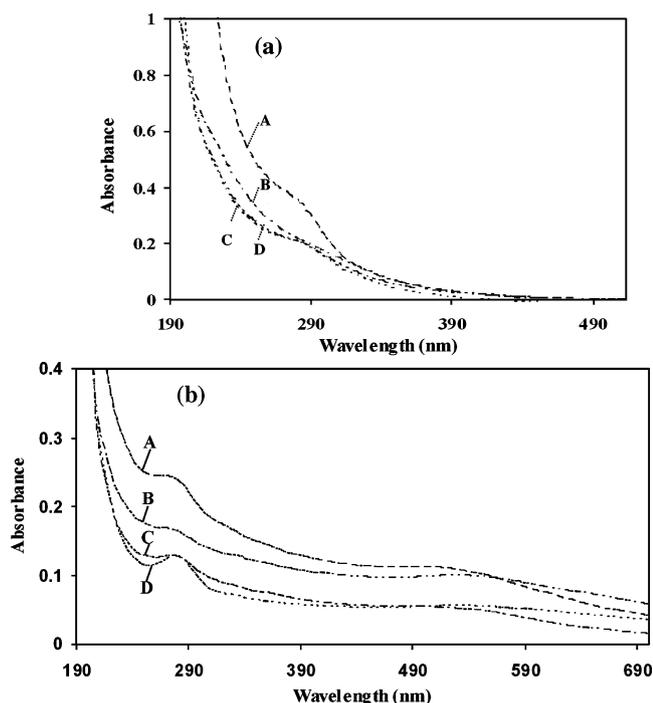


Fig. 1 Absorption spectra of 0.055 mM HAuCl₄ in the presence of 0.05 mM different PAMAM dendrimers, (A) PEG-G3, (B) G3, (C) QG4, (D) G4, before (a) and after (b) reduction with NaBH₄

amino groups of the dendrimer have electrostatic interaction with dissociated AuCl₄⁻ ions. The related UV-Vis spectrum revealed a strong absorption band at 220 nm and a shoulder at 280 nm. The broad band centered on 280 nm was the characteristic of the ion pair formation between AuCl₄⁻ and dendrimer. After reduction by NaBH₄, the brown color of the solutions and UV-Vis spectra (Fig. 1b) both indicated the formation of gold colloids. Surface plasmon resonance (SPR) peak of AuNPs was observed at 520 nm. This was in agreement with the theoretical simulation of SPR using Mie's theory [22]. The absorption band at 280 nm corresponded to certain gold cluster, although Mosseri et al. [23] noted a peak around 320 nm of some gold clusters. The pH of the solution increased to basic after the reduction step. In this situation, it seems that the surface of obtained gold nanoparticles could interact strongly with the amino groups of the dendrimer [24].

Figure 4 illustrates the TEM images and particle size distribution of gold particles. The average diameter of gold nanoparticles for QG4, PEG-G3, and PAMAM G4 and G3 are 6.08, 9.70, 13.90 and 15.37 nm, respectively. The size of gold particles obtained here are bigger than those of PAMAM dendrimers, it is conceivable that the gold dendrimer nanocomposites are formed by dendrimer molecules adsorbing on the gold particles. It is implied that gold particles are stabilized by multiple, surface adsorbed dendrimers, not encapsulated within single dendrimer.

However, the average particle size of gold nanoparticles formed by recruitment of QG4 is smaller than that of intact PAMAM G3, G4 or even PEG-G3. It can be attributed to the fact that QG4 can provide the strongest interaction with a gold nanoparticle and effectively limit particle growth.

To confirm the interaction between gold nanoparticles and dendrimers, fluorescence spectra of dendrimers alone and gold-dendrimer complexes were recorded (Fig. 2a, b). All structures of dendrimers with a concentration of 1 μM showed fluorescent emission. The excitation and emission maximum wavelengths of dendrimers (QG4, PEG-G3, PAMAM G4 and G3) were around 306 and 328 nm. In the presence of AuNPs, the maximum excitation and emission wavelengths shifted to 323 and 353 nm. However, their fluorescence spectra were different. This is a consequence of the strong interaction between AuNPs and the internal amino groups of PAMAM dendrimers. The AuNPs that were protected and reduced by trisodium citrate did not show any fluorescence emission but dendrimer protected AuNPs showed fluorescence emission, suggesting that the dendrimer-stabilizers contribute to the fluorescence properties of the formed Au DNCs. The fluorescence intensities of Au DNCs were weaker than those of dendrimers. In all cases, the Au DNCs and dendrimer-stabilizers are different materials that do not necessarily follow a strict correlation in terms of their relative fluorescence intensities.

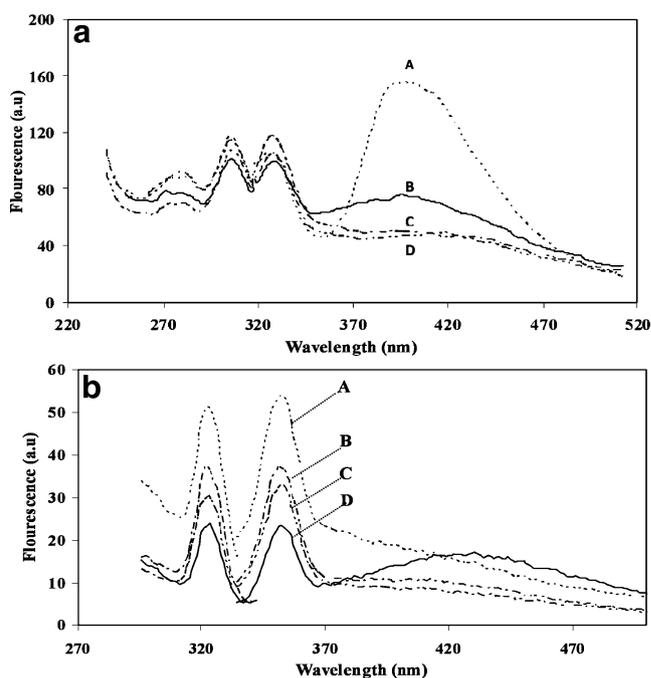


Fig. 2 The excitation (EX) and emission (EM) fluorescence spectra of 0.050 mM of different PAMAM dendrimers, (A) G3, (B) QG4, (C) G4, (D) PEG-G3, (a) alone (b) in the presence of 0.055 mM HAuCl₄ after reduction with 0.066 mM NaBH₄

The effect of gold ion to dendrimer molar ratios on the synthesis of nanocomposites

It is obvious from the literatures that the sizes of nanoparticles can be affected by the concentration of the reactants [25]. The relationship between the particle size of the formed gold nanoparticles and the $[\text{Au}^{3+}]/[\text{PAMAM G4}]$ molar ratio was evaluated using different ratios of those from 0.4:1 to 3:1. The intensity of surface plasmon band of AuNPs in 520 nm increased and shifted to a slightly higher wavelength (Fig. 3). This indicated that the amount of the formed AuNPs could be affected by the $[\text{Au}^{3+}]/[\text{PAMAM G4}]$ mole ratio. This shift also supports an increase in the average AuNPs diameter that is in agreement with the previously reported data [26, 27].

The effect of using different concentrations of PAMAM G4 dendrimer on the absorbance intensity of prepared AuNPs was investigated. It was found that the concentration of dendrimer alone had no obvious effect on the peak intensity of the surface plasmon band of the formed nanoparticles at 520 nm and the mean particle size of those.

The effect of reducing agent on the resulted nanocomposites

The reaction rate i.e., the mass transfer and growth in comparison to nucleation was optimized by different addition rate of reducing agent (NaBH_4 in 0.3 M NaOH). To form tiny nanoparticles, the NaBH_4 should be added quickly while the solution strongly stirred; otherwise the precipitates would be formed in the reaction mixture.

Moreover, the amount of reducing agent was optimized. The result showed that 10-fold molar excess of NaBH_4 was sufficient to completely reduce the Au ions to zero valent Au inside or outside of the dendrimers. It seems that the

precipitation could be occurred in the higher concentration of reducing agent. The nanoparticle aggregation results in the disappearance of the characteristic surface plasmon resonance of nanosized particles.

The effect of PEGylation of PAMAM G3 on the size of Au nanoparticles

The effect of the attached PEG chains on the formation of the nanocomposite and nanotemplating behavior of PAMAM G3 were investigated (Fig. 4). The results showed that the PEG-G3 has better efficiency in comparison to the PAMAM G3 as a nanotemplate, after reduction of gold ions by the optimized amount of reducing agent in the solution. Using PEG-G3 as nanotemplates resulted in smaller nanoparticles that are because of this fact that the shell of PEG chains isolates individual dendrimer molecules from each others and discourages the formation of multi-dendrimer clusters. Therefore the inter-dendrimer coarse gold particle formations were decreased and the resulted product has a good size distribution with a lower SD in comparison to the other tested dendrimers.

Quaternization of PAMAM G4

Bifunctionalized PAMAM G4 having both quaternary and primary amino groups on their interior and periphery, respectively, were synthesized according to the optimized previously reported method [17]. AuNPs were formed in this semi-quaternized PAMAM G4 via diffusion of gold ions into the dendrimer molecules followed by chemical reduction of those in situ. The higher positive charges on the surface of these dendrimers reduced the likelihood of aggregation and the growth of the dendrimer nanocomposites (DNCs). Transmission electron microscopy images revealed that AuNPs had an acceptable polydispersity and a mean diameter of about 6 nm (Fig. 4).

Zeta potential measurements confirmed that the synthesized DNCs were positively charged, with the greatest zeta potentials for QG4 (46.9 mV). Higher zeta potential of nanocomposites of QG4 in comparison to the intact dendrimers indicated that after the formation of the hybrid nanostructures, some of terminal quaternary amines of dendrimers were still available that could prevent the aggregation of the nanocomposites.

Conclusion

It is demonstrated that the dendrimers can be used as an efficient stabilizer and templating agent for the preparation of gold nanoparticles like any other linear polymers.

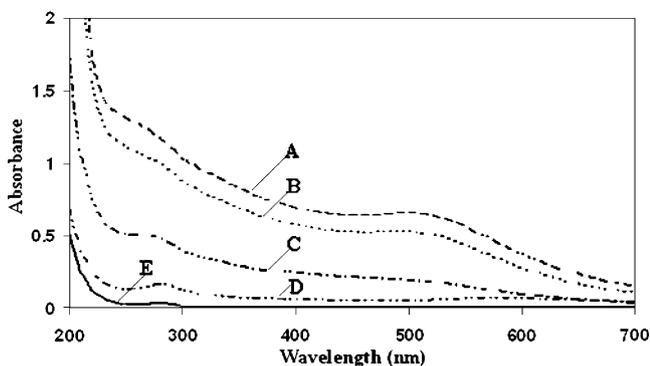
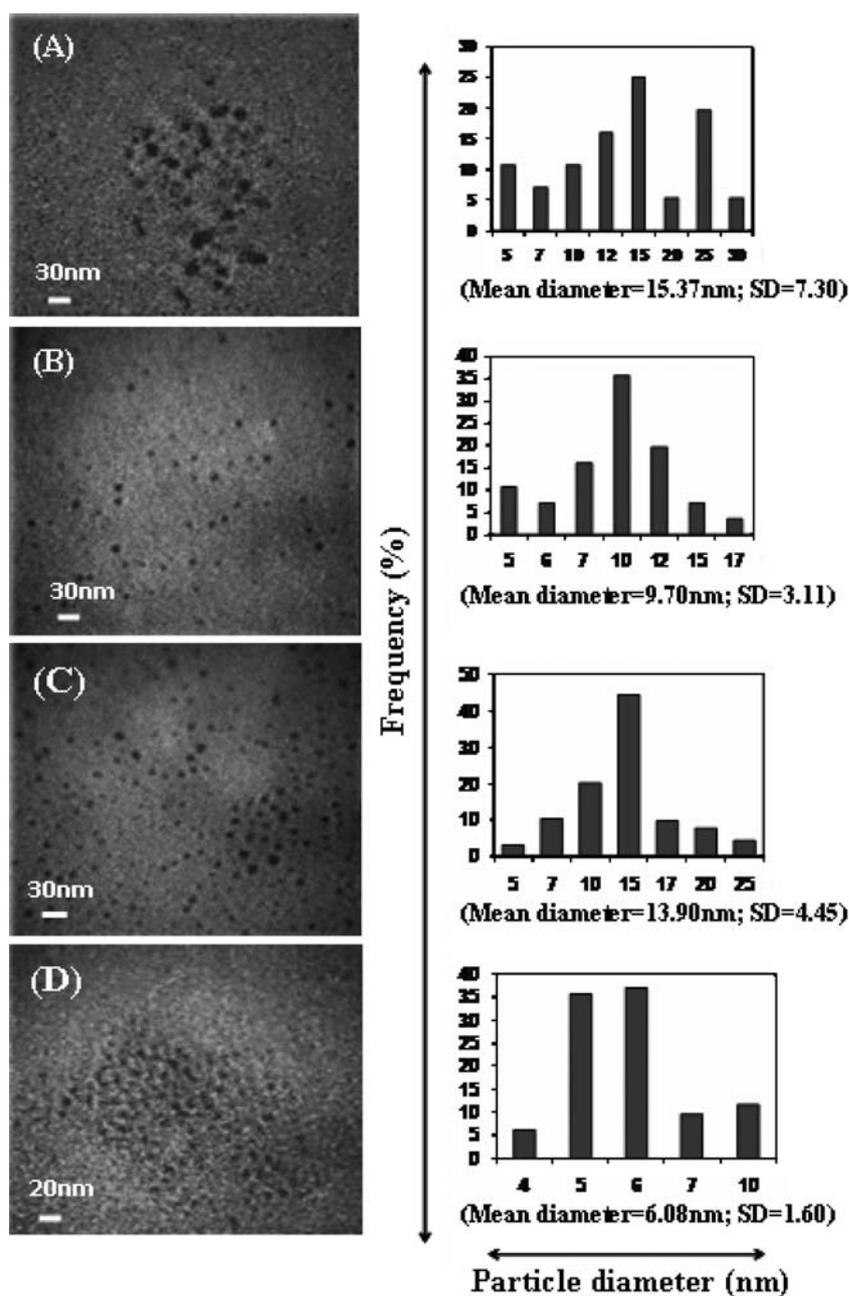


Fig. 3 Absorption spectra of various molar ratio of HAuCl_4 to G4 PAMAM dendrimer (A) 3.0:1, (B) 2.3:1, (C) 1.5:1, (D) 0.8:1, (E) 0.4:1, after reduction with NaBH_4

Fig. 4 TEM micrographs and particle size distribution of gold particles obtained in the presence of various generations of PAMAM dendrimers: (A) G3, (B) PEG-G3, (C) G4, (D) QG4



But it is important to be noted that the surface of the dendrimers such as PAMAM could be tuned for any purposes and applications easily. The generations and the shape of the dendrimers play an important role for the preparation of metal nanoparticles. The Au particle size does appear to be related to dendrimer generation. PAMAM G4 stabilized smaller Au particles compared to PAMAM G3. The improvement in physical properties obtained by modifying the endgroups of PAMAM dendrimers facilitated the development and processing of nanocomposite materials.

In this study, the stabilization of AuNPs by PAMAM dendrimers G3, G4, PEG-G3 and QG4 was evaluated. It could be concluded that the properties of PAMAM G3 have been improved by grafting PEG onto PAMAM G3. In this case, isolating the individual dendrimers in a shell of PEG chains discourages the formation of multidendrimer clusters. The protection properties of PAMAM G4 have been improved by quaternization of primary amines in the periphery of dendrimer. It prevented the aggregation and precipitation of the synthesized dendrimer nanocomposites via spatial repulsion while the nanoparticles formed.

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