pH Effect of Gold Nanoparticles Synthesis Encapsulation in Polyamide Amine Generation 3.0 (AuNP-PAMAM G3.0)

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Abstract
The unique properties of gold nanoparticles (AuNP) allow them to be used as drug and gene delivery agent, so they are effective in diagnosis and therapy. At the moment, one of AuNP development is AuNP encapsulated polyamide amine third generation (AuNP-PAMAM G 3.0). The synthesis of AuNP-PAMAM G 3.0 can be done by reacting HAuCl₄ with PAMAM G3.0, then adding NaBH₄ as a reducing agent. This makes AuNP-PAMAM G 3.0 to be strongly influenced by the pH reaction.

The results of this study show that AuNP has sizes ranging from 11 to 19 nm at pH 2 while their sizes are 2 to 5 nm at pH 7 and 9. The separation using Sephadex PD-10 column shows that the elution efficiencies are 10% at pH 2, 40% at pH 7 and 50% at pH 9. Gold nanoparticles at pH 7 and 9 are more stable than pH 2 in saline. Based on the above results, it is shown that AuNP could be encapsulated by PAMAM G3.0 at pH 7 and 9 but AuNP at pH 2 could not.

Keywords: Gold Nanoparticle (AuNP), PAMAM G3.0, pH, encapsulation.

Introduction
Gold nanoparticles (AuNPs) have unique properties that may be applicable to drug delivery agents or gene delivery agents[1]. The unique properties of AuNPs are their optical and electronic characteristics which are influenced by AuNP sizes, so they can be used in therapeutic and bioimaging fields[2-4]. The AuNP manufacturing is done by reducing $[AuCl_4^-]$ ions with a reductant and added stabilizer to maintain their stability. The development of AuNP as a therapy and drug delivery agent still needs an innovation from several proceeding or supporting studies. Previous studies indicated that AuNP encapsulation in PAMAM has been widely developed to improve the AuNP's ability[5-7]. In the said studies, they were using raw materials from $HAuCl_4$ or $NaAuCl_4$ which are readily available in the market. The AuNP encapsulated PAMAM research in Indonesia has been developed from gold metal (Au Foil) converted into $HAuCl_4$ which is reacted with PAMAM G3 and G4[8-10].

The pH influence in AuNP encapsulated PAMAM synthesis however, has not been observed. The pH effect study is important because PAMAM dendrimers are branched polymer and composed of tertiary amine groups in which the stability is controlled by pH[11]. The stability could pose an effect on the PAMAM's ability to encapsulate the gold nanoparticles (AuNP). The research that proves the pH effect on the AuNP encapsulated PAMAM dendrimer G3.0 synthesis is still limited. Therefore, we will investigate the pH effect on AuNP encapsulated PAMAM by UV-Vis Spectrophotometer, stability in saline solution and separation using Sephadex column PD-10.

Material and Methods
Materials: Polyamidoamine or PAMAM dendsrimers, Ethylene diamine (EDA) core group, Amine surface 3th generation (G3.0) in the form of 10% solution in methanol were purchased from Sigma-Aldrich while Au foil (gold foil) was obtained from Aneka Tambang, the $H^{198}AuCl_4$ solution of Center for Radioisotopes and Radiopharmaceuticals Technology-BATAN. Hydrochloric Acid, nitric acid and sodium borohydride ($NaBH_4$) were purchased from Merck. Water and saline were obtained from IPHA. Whatmann Paper no. 1, inner and outer capsules were from local supply. Sephadex PD-10 column was from GE Healthcare. The beaker glass and Erlenmeyer were obtained from Pyrex, while adjustable micropipettes were produced by Eppendorf. Glass chamber and syringe were purchased by local supplier.

The instruments used in this study were analytical scales (Kern), hotplate magnetic stirrer (Cimarec), radioactivity enumerator (Gammatec II) and Gamma Ionization Chamber (Atomlab Plus) and Spectrophotometer UV/Vis (Jasco32 V-550) and Transmission Electron Microscopy (TEM JEOL JEM 1400 at Chemistry Department, Faculty of Mathematics and Natural Sciences, Gadjah Mada University.

Methods
Preparation of materials targets and irradiation processes: The Au foil was inserted into quartz tube irradiation quality followed by the welding process. The quartz tube was inserted into the inner capsule, then inserted into an outer capsule. Both were made from Aluminium. The capsule tube was delivered to the G.A Siwabessy Reactor

Research Journal of Chemistry and Environment_____Vol. **22(Special Issue II)** August **(2018)**

Res. J. Chem. Environ.

facility to be irradiated at the rabbit system during a controlled time interval (recorded).

Preparation of HAuCl4 from Au foil: In addition, treatment involves the dissolving process of the Au-natural metal using aqua regia to obtain AuCl4-. Gold metals are dissolved in 5 mL of aqua regia (HCl: HNO3) and heated until the solution was evaporated. Then, it is dissolved in 10 mL of aquabidest and the solvent is evaporated. The washing process was done for 3 times. Finally, HAuCl4 was dissolved using aquabidest, so that the concentration of HAuCl4 solution is obtained. The activity of dissolution results was measured using spectrophotometer UV-Vis and Transmission Electron Microscopy (TEM).

Preparation of H^{198}AuCl4 from Au foil (Post-Irradiation Processes): The post-irradiation treatment was done on target material to adjust the processing time since the target material was removed from the irradiation facility. This was also to eliminate possible short-lived radioactive derived from irradiated tube material or targeted material impurities. In addition, the post-irradiation treatment involves the dissolving process of the Au-natural metal using aquaregia to obtain ^{198}AuCl4-. Gold metals were dissolved using 5 mL of aqua regia (HCl: HNO3) and heated until the solution evaporated. Then, it was dissolved in 10 mL of aquabidest until the solvent was evaporated. The washing process was done for 3 times. Finally, the H^{198}AuCl4 was dissolved using aquabidest, so that the concentration of H^{198}AuCl4 solution is obtained. The activity of dissolution results was measured by Gamma Ionization Chamber and the absorbance using UV-Vis Spectrophotometer measurement.

Preparation of gold nanoparticles Au (AuNP) encapsulated PAMAM dendrimer G 3.0: Nanoparticles Au (AuNP) encapsulated PAMAM dendrimer G 3.0 were prepared by reacting PAMAM dendrimer G3.0 with HAuCl4 solution, then stirred for 30 minutes in the flask. NaBH4 0.2 M was added, then stirred for several hours. During the stirring process, the flask was wrapped using aluminium foil. The final pH was measured. In order to encapsulate AuNP with PAMAM Dendrimer G 3.0, the result was investigated with UV-Vis Spectrophotometer at ambient temperature.

Preparation of gold nanoparticles marked ^{198}Au (^{198}AuNP) encapsulated PAMAM dendrimer G 3.0: ^{198}Au (^{198}AuNP) encapsulated PAMAM dendrimer G 3.0 was prepared by the same method followed by purification. The impurities of ^{198}AuNP PAMAM dendrimer G 3.0 were removed using Sephadex PD-10 columns. The characterization was done by UV-Vis Spectrophotometer, Thin Layer Chromatography (TLC) and gamma counter. This method was repeated using HAuCl4 solution at pH 2 and 7.

Gold Nanoparticles PAMAM G 3.0 (AuNP PAMAM G 3.0) Stability in Saline: 0.5 mL AuNP PAMAM G 3.0 pH 2, 7 and 9 solutions were diluted in 1 mL saline. The

solutions were stored in an incubator at 37°C for 7 days. The stability was then measured using UV-Vis Spectrophotometer and visual observation.

Results and Discussion

As shown in figure 1. A, HAuCl4 solution was added to the PAMAM G3.0 solution showing the same color. After adding the NaBH4 solution, the color changed from clear to brownish yellow as shown in figure 1 B. This change occurred due to the reduction process of AuCl4- into gold nanoparticles (AuNP) with a charge of 3$^+$ to 0.

Figure 1: A. Mixed solution of HAuCl4 and PAMAM G3.0, B. Mixed solution of HAuCl4 and PAMAM G3 after adding sodium borohydride (NaBH4)

The change was supported by the results of the spectrophotometry measurements at figure 2. The HAuCl4 solution provided maximum absorbance at about 300 nm. This suggests a transition of transfer of the electron shell d^8 due to the presence of 4Cl- ions as the ligand surrounds the Au^{3+} ions to form complex compounds [AuCl4]-. This transition process was commonly called ligand-to-metal-charge-transfer (LMCT) by chloride ions (Cl-) to Au^{3+} ions.

Based on the UV / Vis spectrum of AuNP-PAMAM G3.0 shown, there was a maximum absorption at about 280 nm[12]. This phenomenon indicates that the AuNP belonged to the PAMAM G3.0 cavity so that AuNP is encapsulated in PAMAM G3.0. The presence of an absorption increasing in the range 400 to 600 nm shows surface plasmon resonance due to the AuNP formation from the reduction process [AuCl4]- to AuNP by NaBH4. The above process shows that PAMAM generation 3.0 can be used as a template to control AuNP growth, so that AuNP nanoparticles would be encapsulated in PAMAM dendrimer G3.0.

AuNP encapsulated in PAMAM G3.0 could stabilize AuNP so they would not be agglomerated. The ability to encapsulate and stabilize AuNP was strongly influenced by the pH reaction and conditions between HAuCl4 and PAMAM G3.0. The PAMAM structure contains a tertiary amine group on the inside of the PAMAM structure. The

*Research Journal of Chemistry and Environment*_____Vol. **22(Special Issue II)** August **(2018)**

Res. J. Chem. Environ.

amine group resulted from the electrostatic force that affected the stability of the amine group during pH change. Figure 3 shows the UV-Vis spectrum and the color of AuNP-PAMAM G3.0 at pH 2, 7 and 9. Both AuNP-PAMAM G3.0 at pH 9 and 7 have similar UV-Vis spectrum and color. The maximum absorbance at about 280 nm was AuNP encapsulated in PAMAM dendrimers G3.0 characteristic.

The presence of absorbance at around 400 to 600 nm was indicating the surface plasmon resonance properties of AuNP encapsulated by PAMAM dendrimers G3.0. However, AuNP-PAMAM dendrimers G3.0 at pH 2 was not found when the maximum uptake was at about 280 nm wavelengths. There was a maximum absorption at 520 nm which showed the surface plasmon resonance properties of AuNP that were not encapsulated by PAMAM dendrimers G3.0. Differences in UV-Vis spectrum forms could be attributed to pH 9 and 7 which are more stable because tertiary amine properties bind the $[AuCl_4]^-$ group and eventually form the gold nanoparticles (AuNP) encapsulated PAMAM G3.0.

After the addition of $NaBH_4$, there is no repulsive in the positive charge force rejecting between the amine structure on the PAMAM surface with the tertiary amine structure inside the PAMAM so it facilitated the AuNP insert into PAMAM G3 cavity. At pH 2, the natural characteristic of tertiary amines on PAMAM was less stable or has weak base properties. Unbinding $[AuCl4]^-$ ion in PAMAM dendrimers restrained the AuNP from entering into the PAMAM G3.0 cavity. PAMAM G 3.0 only served as stabilizer at pH 2.

The differences were also supported by the different color of AuNP-PAMAM solution G3.0 at pH 9, 7 and 2 where the color of AuNP-PAMAM solution G3.0 pH 9 and 7 is brownish. It is identical to the size of the small AuNP nanoparticles. The pink color of the AuNP PAMAM dendrimers G3.0 at pH 2 indicates the larger size of nanoparticles. The morphological measurements of the encapsulated and unencapsulated AuNP using TEM are shown in figure 4. The TEM results show that AuNP at pH 2 has sizes ranging from 11 to 19 nm while AuNP at pH 7 and 9 has sizes ranging from 2 to 5 nm.

The encapsulation result shows that Au Nanoparticles at pH 2 are unencapsulated by PAMAM dendrimer, whereas Au nanoparticles at pH 7 and 9 can be encapsulated by PAMAM dendrimer as shown in figures 5-7. [198]AuNP-PAMAM G3.0 was marked with radioisotopes. The [198]Au at pH 9 and 7 shows the eligible efficiency elucidated from [198]AuNP-PAMAM G3.0 which was about 50% after being passed in Sephadex PD-10 columns. The columns at pH 7 and 9 showed clear color. It means that all AuNP passed. The [198]AuNP-PAMAM G3.0 marked that radioisotope [198]Au with pH 2 has an efficiency of below 10%. These results indicated that AuNP at pH 2 was not encapsulated in PAMAM G3.0 which caused precipitation in the Sephadex PD-10 column. The column colour became purple.

Based on figure 8, different pH of AuNP-PAMAM dendrimers G3.0 absorb radiation of different wavelengths. Indirectly, the absorption bands correspond to pH. The results of the UV-Vis spectrophotometer measurements at pH 2 indicated the absence of surface plasmon resonance properties at a wavelength of about 400 to 600 nm while at pH 7 and 9, they were present. All samples have an absorbance at about 280 nm which indicates that AuNP are still encapsulated in PAMAM dendrimers G 3.0.

The absence of surface plasmon resonance in AuNP-PAMAM G3.0 pH 2 was related to unstable AuNP because AuNP unencapsulated PAMAM G3. Its stability was disrupted by Na^+ and Cl^- ion causing AuNP precipitation. AuNP-PAMAM G3.0 at pH 7 and 9 stability was undisturbed by Na^+ and Cl^- ion because PAMAM G3.0 encapsulated AuNP well which serves as stabilizer. The above results show that AuNP-PAMAM G3.0 at pH 2 could not be used for therapy or diagnosis due to AuNP undergoing deposition.
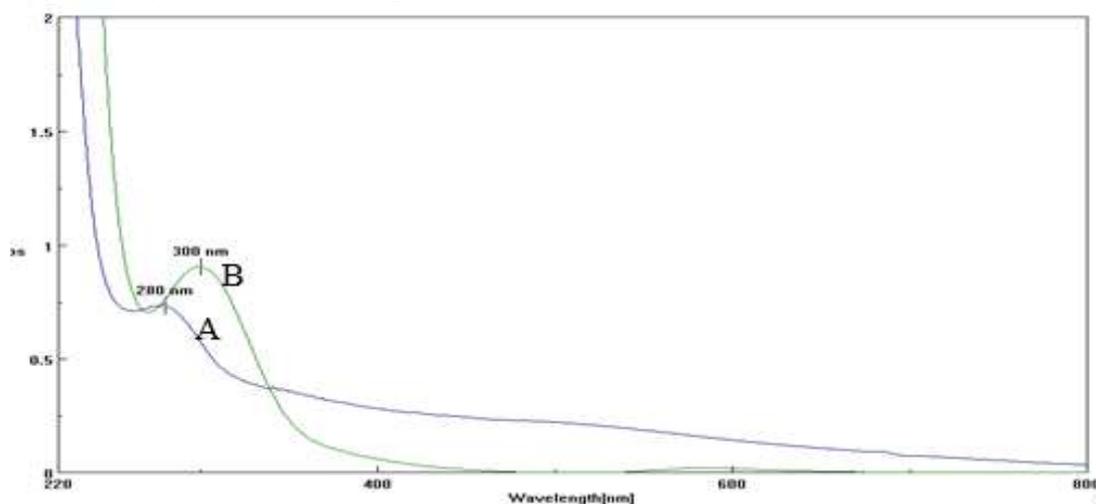

Figure 2: Overlay of the UV-Vis Spectrum: A. Gold nanoparticles (AuNP) encapsulation in polyamide amine (PAMAM) dendrimer generation 3.0 [AuNP-PAMAM G3] and B. HAuCl₄ solution
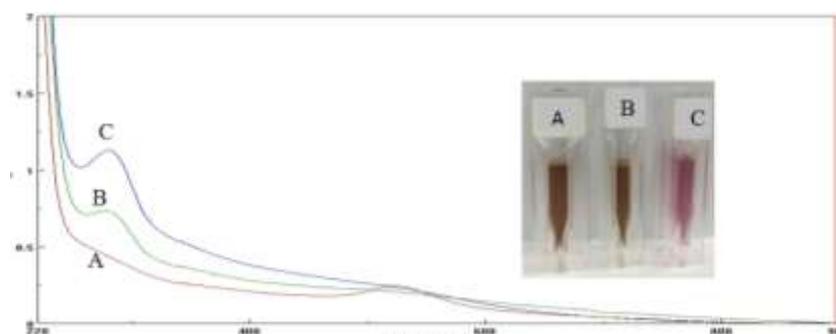
Figure 3: Overlay of gold nanoparticles (AuNP) encapsulation in polyamide amine generation 3.0 (AuNP-PAMAM G3) UV-Vis spectrum at (A) pH 2, (B) 7 and (C) 9
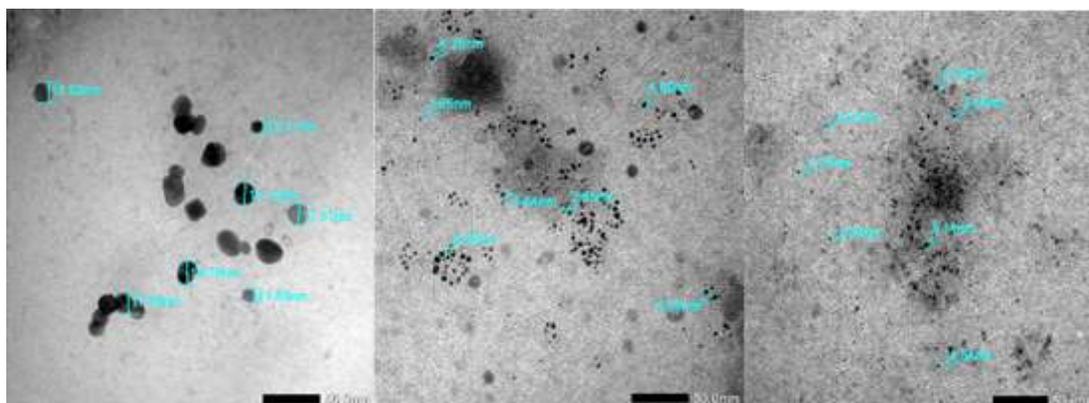

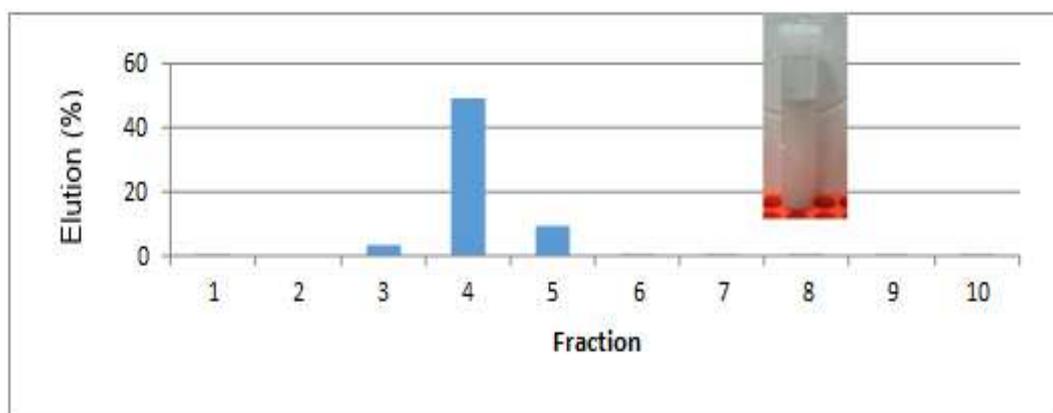**Figure 4: Effect of pH on AuNP size encapsulated PAMAM dendrimer G 3.0**

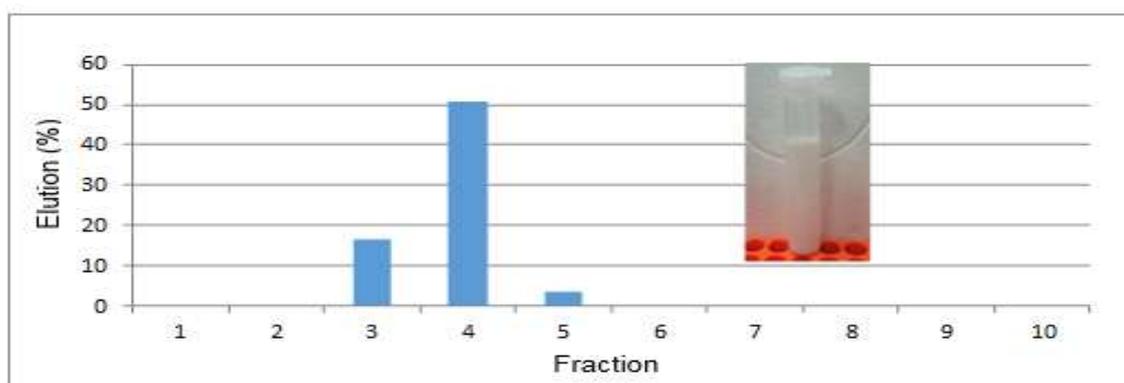**Figure 5: Efficiency of [198]AuNP-PAMAM dendrimers G3.0 pH 9 after passed through the Sephadex PD-10 column**

Figure 6: Efficiency of [198]AuNP-PAMAM G3.0 pH 7 after passed through Sephadex PD-10 column
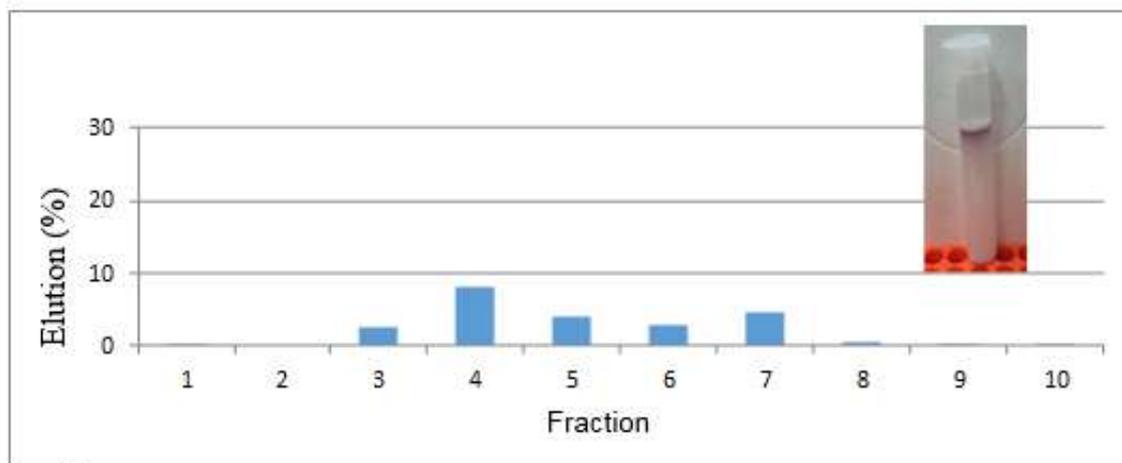
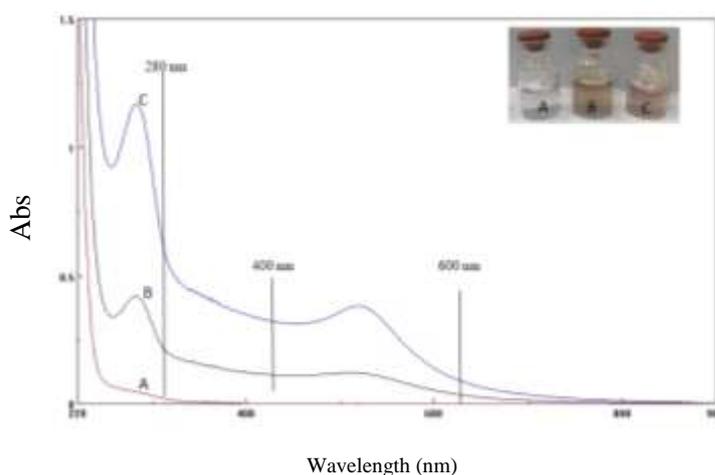**Figure 7: Efficiency of ^{198}AuNP-PAMAM G3.0 pH 2 after passed through Sephadex PD-10 column**

Figure 8: AuNP-PAMAM G3.0 stability at (A) pH 2, (B) 7 and (C) 9 in saline solution after being stored in an incubator at 37°C for 7 days

Conclusion

The pH of AuNP encapsulated PAMAM dendrimers G 3.0 was highly influenced in encapsulation process, size and stability. Based on TEM, it indicates that AuNP at pH 2 has sizes ranging from 11 to 19 nm which is larger than AuNP at pH 7 and 9 with sizes in the range of 2 - 5 nm. The separation result using Sephadex PD-10 column shows AuNP-PAMAM G3.0 at pH 7 and 9 has elution efficiency 40 to 50%, while AuNP-PAMAM G3.0 at pH 2 is below 10%. AuNP-PAMAM G3.0 PH 7 and 9 are more stable than AuNP-PAMAM G3.0 pH 2 in saline solution.

Acknowledgement

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Res. J. Chem. Environ.

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