

Comparison of Different Methods for Purification of Gold Nanoparticles– Polyamidoamine (PAMAM) Generation 4 Dendrimer

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Abstract

Gold nanoparticles (AuNP) are currently being widely applied in cancer diagnosis as a CT Scan contrast agent (imaging) as well as in therapy (theragnostic agents). The polyamidoamine (PAMAM) G4 dendrimer has also been applied as a stabilizer agent to control the size of gold nanoparticles. In the production of AuNP-PAMAM G4 dendrimer, a solvent and reducing agent were used. Therefore, it is necessary to separate and purify the synthesis result of AuNP-PAMAM G4 dendrimer from the undesired compounds.

Separation and purification were carried out by two different methods, namely, size exclusion column chromatography Sephadex G25[®] (SE), and membrane ultrafiltration (MU). The percent yield of AuNP that has been synthesized was calculated by using Inductively Couple Plasma Atomic Emission Spectroscopy (ICP-OES) and then characterized by using UV-Vis Spectroscopy and Transmission Electron Microscope (TEM). The percent yield of AuNP, which was carried out by MU, was 93.75%. This result was better compared to using SE (81.18%). The characteristics results from the UV-Vis spectrum demonstrated similar spectra between separation and purification methods using both MU and SE. The characteristics results from TEM also showed similar morphological results.

Keywords: Contrast agents, CT-Scan, dendrimer, gold nanoparticles, purification.

Introduction

The rapid development of nanotechnology has recently led to research on the synthesis or manufacture of therapeutic agents as well as the diagnosis (theragnostic) on the nanoscale, one of which involves gold nanoparticles. The gold nanoparticles were used as theragnostic agents, attributable to their advantages over the conventional one (iodine). Gold nanoparticles have higher atomic numbers

and electron densities and are not toxic in a large concentration range, and their gold surface is easily modified for targeted delivery¹⁻³. The technology used in the production of gold nanoparticles often involves top-down and bottom-up methods. The bottom-up method involves a stabilizer to prevent aggregation, and as a template for the growth of nanoparticles⁴.

In the production of gold nanoparticles, tetrachloro auric acid (HAuCl₄) is reduced by sodium borohydride (NaBH₄) and PAMAM G4 dendrimer as a stabilizer agent for growing the template and controlling the particle size of the gold nanoparticles. Tetrachloroauric acid was produced by dissolving Au foil using aqua regia/ HCl: HNO₃ (3:1)⁶. The excess of organic solvents and reducing agents must be removed by using some method of separation and purification such as size exclusion column chromatography (SE) and membrane ultrafiltration (MU).

This work involves the comparison of two purification methods of gold nanoparticles PAMAM G4 using SE and MU. The optimal yield will be a consideration to determine the most suitable method for this purification. Furthermore, gold nanoparticles PAMAM G4 dendrimer can be used safely as a cancer theragnostic agent and this approach can be used as a prototype for the manufacture of a larger production scale.

Material and Methods

Materials: Au foil was purchased from PT. Antam (Indonesia), and all chemicals were obtained from Aldrich (USA) and Merck (Germany).

Preparation of HAuCl₄ solution: Au foil was dissolved in aqua regia (HCl: HNO₃ = 3:1). The solution was stirred and heated at 100°C (kept not to burst), and then dissolved with demineralized water. The dissolution with demineralized water and heating process was performed three independent times. Finally, HAuCl₄ was dissolved in 0.01 M HCl solution.

Synthesis and characterization of AuNP-PAMAM G4 dendrimer: The prepared HAuCl₄ solution (0.635 mL) was

added to 1.75 mL PAMAM G4 dendrimer solution. The solution mixture was stirred for 30 minutes and to complete the formation of AuNP-PAMAM G4 dendrimer reduced Au was done by 25 μL NaBH_4 for 4 hours. Then add demineralized water to the desired volume. The absorption spectrum was obtained with UV-Vis Spectrophotometer of encapsulated gold nanoparticles PAMAM G4 dendrimer at 200-800 nm wavelength.

Morphological observations and particle determination were conducted using a Transmission Electron Microscope (TEM). The determination of AuNP-PAMAM G4 dendrimer particle morphology was performed using 1 mL of solution in each test. The determination of the number of AuNP was carried out using ICP-OES.

Purification and Separation of AuNP-PAMAM G4 dendrimer: The purification by using the SE was performed by taking 1 mL of the sample, and then eluting it using PBS until 15 fractions of each fraction of 1 mL were obtained.

The purification by using MU was performed by introducing 5 mL of the sample into MU, which was then centrifuged for 15 min to 1 mL. The remaining sample was taken, and demineralized water was added to a volume of 5 mL. After both methods of the purification and separation of AuNP-PAMAM G4 dendrimer, and comparison of the characterization, the Au content obtained was determined using ICP-OES.

Results and Discussion

The preparation of HAuCl_4 solution was carried out using Au foil and dissolved by using aqua regia in hot conditions to facilitate the dissolution process. The addition of demineralized water was then heated to vaporize HNO_3 contained in aqua regia. The washing process was performed three independent times. This study involved the use of PAMAM G4 dendrimer and reducing NaBH_4 . At the same time of the addition of HAuCl_4 into the dendrimer solution, PAMAM G4, stirring was done for 30 minutes with the aim of the gold being diffused and mixed first with the PAMAM G4 dendrimer. After that, it was reduced to 4 hours with NaBH_4 to obtain AuNP-PAMAM G4 dendrimer in the maximum amount.

AuNP-PAMAM G4 dendrimer was formed by showing different color characters compared to HAuCl_4 . The encapsulated AuNP-PAMAM G4 dendrimer was brown while the AuNP-PAMAM G4 dendrimer which was not encapsulated was blackish purple. The HAuCl_4 was yellow (figure 1).

Based on Sutriyo et al⁶ and Esumi et al⁷, HAuCl_4 had a UV-Vis absorption of about 310 nm. Unencapsulated AuNP with PAMAM G4 dendrimer showed a peak at 530 nm, while the encapsulated AuNP with Pamam G4 dendrimer did not show absorption at 310 nm and 530 nm. This is due to the agglomeration between AuNP. As for PAMAM G4

dendrimer, there was no peak increase at both 312 nm and 530 nm. Based on Kim et al⁸, the appearance of the absorption peak at 530 nm was due to the AuNP being larger than 2 nm whereas in the spectrum that did not show a peak at 530 nm, the particle size was 2-3 nm (figure 2).

The purification and separation of AuNP-PAMAM G4 dendrimer was carried out using SE with PBS (phosphate buffer saline) as an eluent and accommodated to 15 fractions of 1 mL each. The Au content of each fraction was determined by means of UV-Vis spectroscopy. Based on figure 3, the fraction showing the AuNP-PAMAM G4 dendrimer is at fractions 3, 4 and 5. This corresponds to the characteristic AuNP-PAMAM G4 dendrimer which does not show the absorption peak at 530 nm. Meanwhile, based on the determination of AuNP levels using ICP-OES, AuNP only exists in fractions 3, 4 and 5.

A morphological observation was observed using the TEM to ensure the formation of AuNP-PAMAM G4 dendrimer where AuNP encapsulated in PAMAM G4 dendrimer was used as a stabilizer agent. However, if AuNP-PAMAM G4 dendrimer was not formed, the aggregation between AuNP particles caused a larger size than the AuNP-PAMAM G4 dendrimer formed, as seen in figure 4. Based on figure 4, purification using SE (figure 4C) and MU (figure 4D) can be done, because it still has a similar morphology to the AuNP-PAMAM G4 dendrimer that was formed. This can be seen because there is no aggregation between gold nanoparticles.

The determination yields of AuNP-PAMAM G4 dendrimer were obtained using ICP-OES. After obtaining Au content, the percent yield was calculated by using the following formula:

$$\% \text{ yield} = \frac{\text{Au after purification}}{\text{Au before purification}} \times 100$$

Based on table 1, the yield obtained for MU is 93.75% while using the SE obtained amounted to 81.18%. The use of MU generates a greater yield compared to SE, since the sample dilution SE occurs from the use of eluent, and SE can also hold larger particles to exit the column. The use of MU is not possible to separate the larger sized particles from the smaller ones. Therefore, we recommend that the use of MU must be confirmed in advance, the characteristics of AuNP-PAMAM G4 dendrimer are already well-encapsulated with the size of 2-3 nm using TEM and UV-Vis that do not exhibit absorption peaks at 530 nm.

Conclusion

The purification and separation of AuNP-PAMAM dendrimer G4 synthesis results can be carried out using size exclusion column chromatography (SE) and membrane ultrafiltration (MU). The use of membrane ultrafiltration results in a better yield and faster processing time. The characteristics results of both using UV-Vis Spectroscopy and TEM demonstrated good results because there was no difference reported between the two.

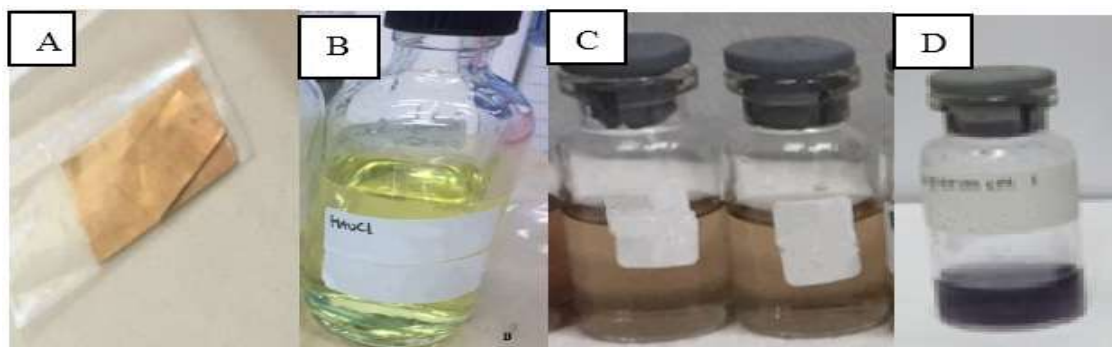


Figure 1: (A) Au foil, (B) HAuCl₄ solution, (C) the encapsulated AuNP-PAMAM G4 dendrimer, (D) the unencapsulated AuNP-PAMAM G4 dendrimer

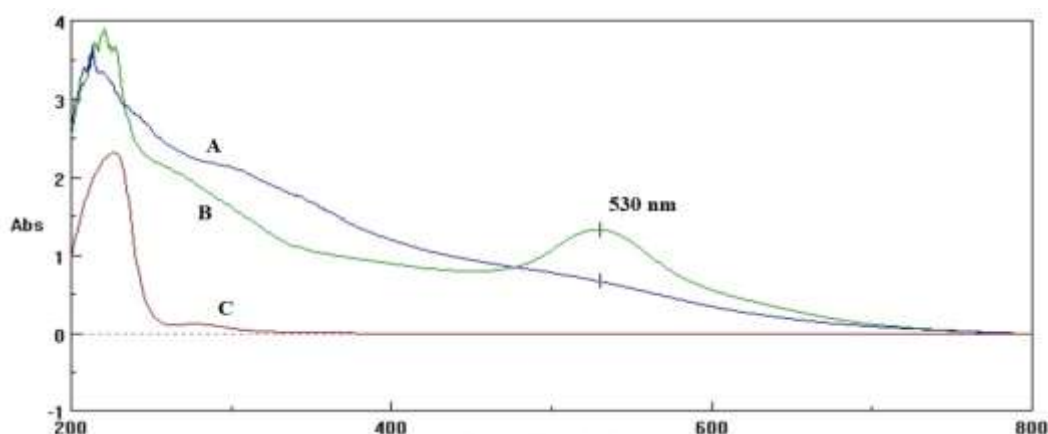


Figure 2: (A) the encapsulated AuNP-PAMAM G4 dendrimer, (B) the unencapsulated AuNP-PAMAM G4 dendrimer, (C) PAMAM G4 dendrimer

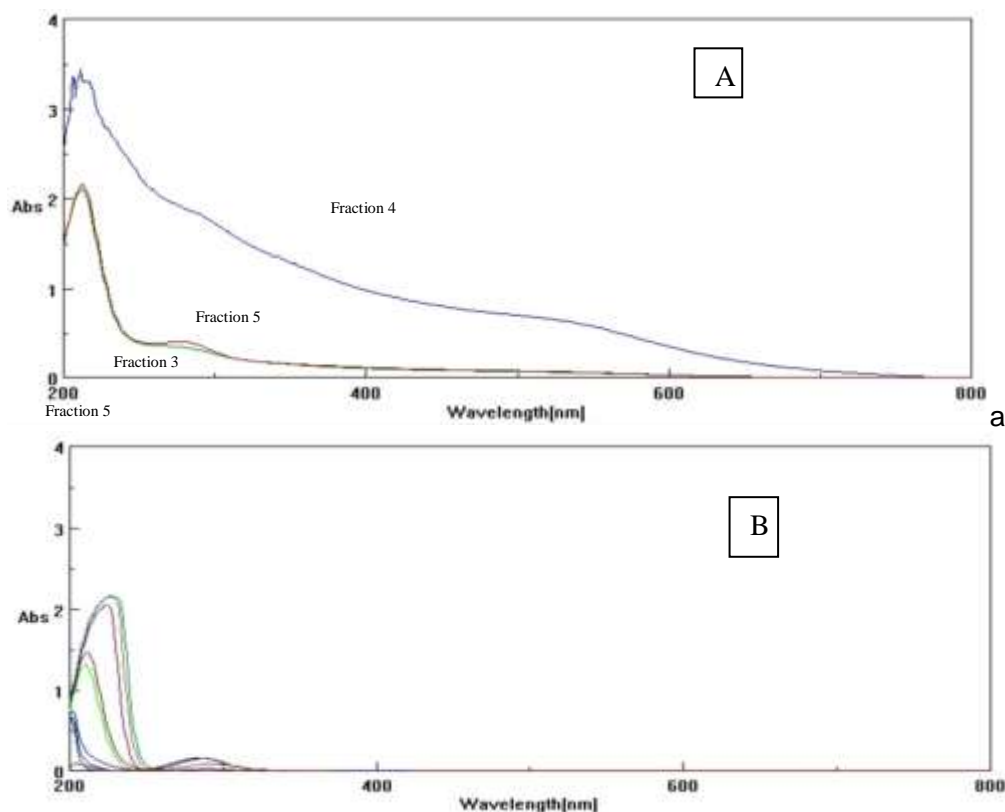


Figure 3: (A) UV-Vis absorption of AuNP-PAMAM G4 dendrimer fraction 3,4 and 5 after purification and separation by SE (B) UV-Vis absorption of AuNP-PAMAM G4 dendrimer fraction1, 2 and 6-15 after purification and separation by SE

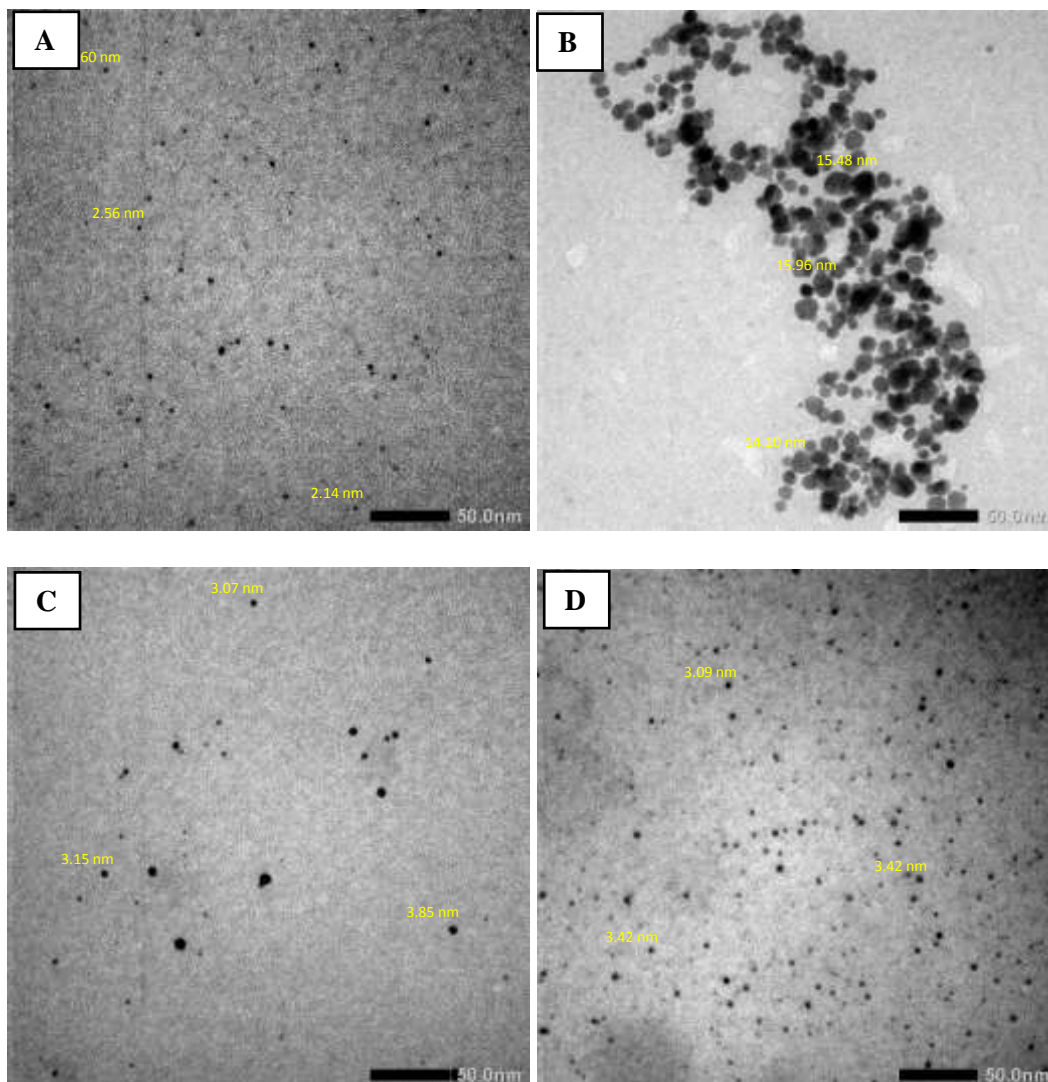


Figure 4: (A) the encapsulated AuNP-PAMAM G4 dendrimer before purification and separation, (B) the unencapsulated AuNP-PAMAM G4 dendrimer, (C) the encapsulated AuNP- PAMAM G4 dendrimer after purification and separation by SE, (D) the encapsulated AuNP-PAMAM G4 dendrimer after purification and separation by MU

Table 1
AuNP- PAMAM G4 dendrimer obtained using ICP-OES

Au before purification (mg/mL)	Au after purification using MU (mg/mL)	% yield
0.032	0.0292	91.2500
0.032	0.0301	94.0625
0.032	0.0307	95.9375
Average		93.75
Au before purification (mg/mL)	Au after purification using SE (mg/mL)	% yield
0.0317	0.0260	82.01893
0.0317	0.0253	79.81073
0.0317	0.0259	81.70347
Average		81.1777

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