Synthesis and Characterization of Poly(Amidoamine) Dendrimers Encapsulated ^{198}Au Nanoparticles

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ABSTRACT

Brachytherapy or internal radiotherapy is one of many methods used for treatment of cancer. This modality requires an agent with radionuclides that emits α or β particle with a proper energy. ^{198}Au (99% β max = 0.96 MeV and $t_{1/2}$ = 2.69 days) is one of radionuclides that has been considered to be effective for the above-mentioned purpose. The purpose of this research was to synthesis and characterize poly(amidoamine) (PAMAM) G3.0 dendrimers encapsulated ^{198}Au nanoparticles as a new brachytherapy agent. PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles was successfully synthesized by a bottom-up method using sodium borohydride as a reductor. Purification was then performed by a size exclusion chromatography in order to separate large Au nanoparticles that were formed outside the cavity of PAMAM G3.0 dendrimers. Prior to the synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles, the synthetic procedure was first established by using a non-radioactive Au. The PAMAM G3.0 dendrimers encapsulated Au nanoparticles produced was then characterized by using an UV-Vis spectroscopy, a transmission electron microscopy (TEM), particle size analyzer (PSA), and an atomic absorption spectroscopy (AAS). Characterization results revealed that PAMAM G3.0 dendrimers encapsulated Au nanoparticles that were prepared from a reaction mixture of PAMAM G3.0 dendrimers and Au $HAuCl_4$ with mol ratio of 2.8, was found to be a proper formula. It produced PAMAM G3.0 dendrimers encapsulated Au nanoparticles with diameter of 1.743 nm, spheris, uniform and drug loading value of 26.34%. This formula was then used in synthesis using radioactive Au, ^{198}Au. Characterization results of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles gave a radiochemical purity of 99.4% and zero charge.

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INTRODUCTION

Cancer is a disease that threatens human life. It has been reported that there were 12 millions of new cancer cases in 2008, with the number of deaths of 7.5 millions of 6 billions total world cancer population [1]. Meanwhile in Indonesia in 2008 there were a number of 292.6 thousands new cancer cases with the number of death of 21.4 thousands of 227 millions Indonesia cancer population [1]. Therefore a lot of attention for curing the disease has gained every single year.

Radiotherapy is one of cancer treatment methods that has been resulting different of therapeutic success [2]. It is based on the use of radiation which is emitted by radioactive substances.

One type of radiotherapy is internal radiotheraphy which is also known as brachytherapy. Brachytherapy could be used to treat various types of solid cancers, such as prostate, cervix, breast, and others. Brachytherapy is performed by placing radioactive material inside or near the tumor temporaly or permanently using a catheter [3]. Brachytherapy is generally perfomed by using micro-sized radioactive seed (50-100 μm) made of ^{125}I, ^{103}Pd or ^{90}Y (Therasphere ™) [4].

Radionuclides of gold, ^{198}Au [4], has been used for brachytherapy since 1950 [2]. This

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application is due to its physical properties that are attractive for the above-mentioned purpose. ^{198}Au with a half life ($t_{1/2}$) of 2.7 days, emits β particle (99% β max = 0.96 MeV) which is ideal for radiotherapy of cancer. It also emits γ radiation; (0.98% γ = 1.1 MeV), which is also ideal for dosimetry, pharmacokinetics and imaging studies [5]. It has been reported by Loening that a direct delivery of radionuclides ^{198}Au to cancer cells was found to be quite successful in eradicating cancer cells [6], however the delivery was constrained by the limited doses of ^{198}Au activity which was able to be deposited inside or near cancer cells. This limitations could be overcome by encapsulating a number of ^{198}Au nanoparticles inside the cavity of dendrimers.

One of the ideal drug delivery carriers that has been widely used is dendrimer [5]. This substance is a macromolecule that has nano structures [7], globular-shaped, has monodispersity in size, and functional groups on its surface [8]. Dendrimer structure consists of three main parts, namely the structure of the nucleus (core), branching (generation), and surface functional groups [9]. The advantages of dendrimer beside its nano-scale size is its ability to encapsulate an organic or inorganic molecules and metal nanoparticles that is referred to an unimolecular encapsulation in its internal interior or known as cavity [10]. P(olyamidoamine) dendrimer- Ethylene Diamine (EDA) core with amine functional groups (PAMAM) dendrimers is a type of dendrimers that has been widely used as drug delivery agent in the biomedicine field because of its biocompatibility and low immunogenicity [8,11]. The 3rd generation PAMAM (G3.0) is known to have characteristics that suitable for in vivo application [12]. Structure of PAMAM G3.0 dendrimer can be seen in Fig. 1 [13].

PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles is one example case of unimolecular encapsulation method. The advantage of this method is that the outer layer of PAMAM G 3.0 dendrimer does not change so that the outer layer can still interact with body tissues. Therefore by this method, the radioactivity dose of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles can be delivered to the target (cancer cells) effectively.

^{198}Au nanoparticles consist of at least 20000 of Au atoms that can kill and destruct cancer more powerful [14]. Dendrimers have also been widely used as a template to control size and dispersity of Au nanoparticles as well as play role as a stabilizer in the synthesis because Au nanoparticles tend to agglomerate [15-17].

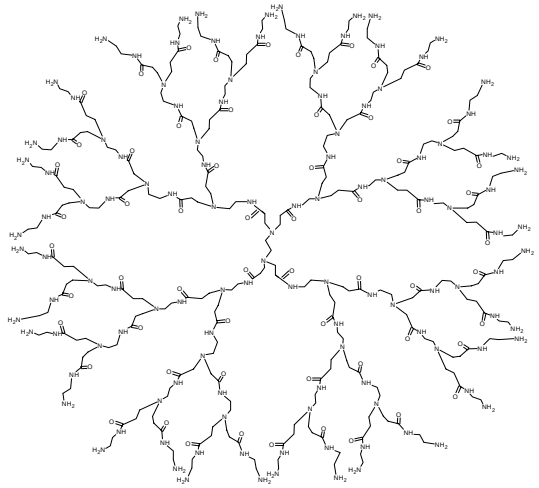

Fig. 1. The structure of PAMAM G3.0 dendrimers[13].

Preparation of PAMAM dendrimer encapsulated nanoparticles ^{198}Au was reported can be obtained by two methods [5]. Firtsly by irradiating the prepared PAMAM dendrimer encapsulated Au nanoparticles where PAMAM dendrimer encapsulated Au nanoparticles is prepared by reacting non radioactive (*cold*) Au (Au) in form of $[AuCl_4]^-$ with PAMAM dendrimers which then followed by a reduction of Au (III) into Au (0) nanoparticles by addition of sodium borohyride ($NaBH_4$) [5]. Secondly, by reacting a $[^{198}AuCl_4]^-$ with dendrimers where $[^{198}AuCl_4]^-$ was obtained from irradiating gold foil [5] in a nuclear reactor facility. Similar to the above-mentioned, PAMAM dendrimers encapsulated ^{198}Au nanoparticles also undergoes reduction process by addition $NaBH_4$ as a reductor to change Au (III) into Au (0) nanoparticles [15]. In order to obtain an acceptable PAMAM dendrimers encapsulated ^{198}Au nanoparticles a purification process is performed. A common method for purification of PAMAM dendrimers encapsulated ^{198}Au nanoparticles is by using a size exclusion chromatography (SEC) [18,19].

The purpose of this study was to synthesis of PAMAM G3.0 dendrimer encapsulated ^{198}Au nanoparticles which was expected to be used as brachytherapy agents for cancer theraphy, particularly for prostate cancer. In this study PAMAM G3.0 dendrimer encapsulated ^{198}Au was prepared in similar way with the reported method, by reacting a $[^{198}AuCl_4]^-$ [2] and PAMAM G3.0 dendrimers. The resulted product was then undergoing reduction process by addition $NaBH_4$ as a reductor to change Au (III) into Au (0) nanoparticles [15]. Purification of PAMAM G3.0 dendrimer encapsulated ^{198}Au was performed

by using a commercial SEC column PD-10 (Sephadex G25).

EXPERIMENTAL METHOD

Materials used in this study included PAMAM G3.0, bovine serum albumin (BSA), gold chloride ($HAuCl_4.3H_2O$), methanol, and Au foil were obtained from Sigma-Aldrich. Hydrochloric acid, nitric acid, sodium borohidride ($NaBH_4$), sodium phosphate dibasic ($Na_2HPO_4.2H_2O$), sodium phosphate monobasic ($NaH_2PO_4.H_2O$), and Whatman paper No 1 were purchased from Merck. Aquabidest and saline solution were obtained from IPHA, and PD-10 column (contain Sephadex G25 medium) was from GE Healthcare. Au-198 foil was prepared by irradiating of Au foil at the G.A. Siwabessy, Multi Purposes Reactor.

Equipment used in this study were *hotplate magnetic stirrer* (Cimarec), UV-Visible spectroscopy from (Jasco V-550), Atomic Absorption Spectroscophy AA 6300 (Shimadzu), dose calibrator (gamma ionisation chamber) (Atomlab100 plus Biodex Medical System), Transmission Electron Microscopy (TEM) JEM 1400 (JEOL Ltd), electrophoresis EC 1000 XL (Thermo Scientific), Single Channel Analyzer (Bioscan), and Zeta Sizer Nano ZS (Malvern).

Methods

Prior to synthesis of PAMAM G3.0 dendrimers encapsulated radioactive Au (^{198}Au) nanoparticles, the synthesis was established by performing a non active procedure.

Preparation of $HAuCl_4$

Gold foil (20.1 mg) was dissolved in 5 mL aqua regia, the solution was then heated and stirred untill almost dried. It was then redissolved with 10 mL water, reheated with stirring untill almost dried (these steps were repeated three times). Finally, the residue was reconstituted in aliquot of 0.01 M HCl to give 0.002 M $HAuCl_4$. The solution was then characterized using UV-Vis spectroscopy.

Synthesis of PAMAM G3.0 dendrimers encapsulated Au nanoparticles

The methods used similar to that of reported by to Ezumi *et al*. [15] with some modifications. A series of $HAuCl_4$ solution, consisted of 0.002; 0.001; 0.0005 M $HAuCl_4$ and a series of PAMAM G3.0 consisted of 0.0014 and 0.0028 M PAMAM G3.0. The reactions were performed by addition of $HAuCl_4$ to PAMAM G3.0 solutions to give reaction mixtures as follow: F1 (0.0014 M PAMAM G3.0 + 0.002 M $HAuCl_4$ solution , to give a final molar ratio of PAMAM to $HAuCl_4$ = 0.7); F2 (0.0014 M PAMAM G3.0 + 0.001 M $HAuCl_4$ solution, to give a final molar ratio 1.4); F3 (0.0014 M PAMAM G3.0 solution + 0.0005 M $HAuCl_4$ solution, to give a final molar ratio 2.8); F4 (0.0028 M PAMAM G3.0 solution + 0.002 M $HAuCl_4$ solution, to give a final molar ratio of 1.4); F5 (0.0028 M PAMAM G3.0 solution + 0.001 M $HAuCl_4$ solution, to give a final molar ratio of 2.8); and F6 (0.0028 M PAMAM G3.0 solution + 0.0005 M $HAuCl_4$ solution, to give a final molar ratio of 5.6). The reaction mixtures were left to react for 15 mins by a stirring at room temperature and protected from light. Aliquot amount of 0.02 M $NaBH_4$ solution was then added to the reaction mixtures which were then left to react for 48 hours. The reaction mixture was then characterized using UV-Vis spectroscopy.

Purification of PAMAM G3.0 dendrimers encapsulated Au nanoparticles

PAMAM G3.0 dendrimers encapsulated Au nanoparticles was purified from larger Au (> 2 nm) nanoparticles that was formed outside PAMAM G3 cavity using PD-10 column. The PD-10 column prior to its use were pre-blocked and pre-equibrated with 1 mL of 0.5% BSA and.01 M PBS pH 7.5 respectively. One mL of PAMAM G3.0 dendrimers encapsulated Au nanoparticles sample was loaded onto the top of the column. The column was then eluted with 0.01 M PBS pH 7.5 and fractions (1 mL/ fraction) were retrieved. About 10 fractions were collected and characterized using a Biorad dye.

Determination of the fraction containing PAMAM G3.0 dendrimers encapsulated Au nanoparticles

In order to determine which fraction containing PAMAM G3.0 dendrimers encapsulated Au nanoparticles, a 10 µL from each fraction was pipet out and transferred into a 96-well plate. To each sample was then to 90 µL of a Biorad dye solution (diluted ¼). The colorless samples which that changed to blue indicated that they contained PAMAM G3.0 dendrimers encapsulated Au nanoparticles. These fractions were then used for further characterization which included morphology, average diameter, and

entrapment eficiency by using TEM, PSA, and AAS, respectively.

Synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles "hot procedure"

Preparation of $H^{198}AuCl_4$ from Au foil.

The Au foils were irradiated in rabbit facility of the GA Siwabessy Multi Purposes reactor, Serpong with 1.2 x 10^{13} n/cm^2/s neutron flux for five hours. Radioactive Au foil (^{198}Au) was treated in the hot cell facility which is similar to the working step for preparation of $HAuCl_4$ solution. Radioactivity of $H^{198}AuCl_4$ was measured with dose calibrator. Radiochemical purity and charge of $H^{198}AuCl_4$ solution were characterized with thin layer chromatography by using a Whatman paper No. 1 as a stationary phase and methanol 70% as mobile phase. The charge of $H^{198}AuCl_4$ solution was measured with electrophoresis by using a Whatman paper No.1 and phosphate buffered 0.01 M pH 7.5 as a stationary phase and phase respectively. The chromatographic profiles of these papers were then measured by using BioScan TLC scanner.

Synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles.

The synthesis of PAMAM was carried out with the same procedure for the synthesis of non radioactive PAMAM G3.0 dendrimers encapsulated Au nanoparticles.

Purification of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles

Purification of ^{198}Au nanoparticles encapsulated PAMAM G3.0 performed in the same way as for non-radioactive PAMAM G3.0 dendrimers encapsulated Au nanoparticles. The sample obtained were analyzed by TLC and electrophoresis. The chromatographic profiles of these were measured using by Bioscan TLC scanner.

RESULTS AND DISCUSSION

Prior to synthesis of PAMAM G3.0 dendrimers encapsulated radioactive gold (^{198}Au) nanoparticles, the synthetic procedure was firstly established by optimizing the non-radioactive procedure. The established procedure was then applied for preparation PAMAM G3.0 dendrimers encapsulated radioactive ^{198}Au nanoparticles. The reason for firstly establishing the non-radioactive procedure was mainly due to the difficulty in characterization of radioactive samples with several systems such as TEM, PSA, and AAS. Therefore, by preparing PAMAM G3.0 dendrimers encapsulated non radioactive Au nanoparticles and establishing its preparation procedure which resulted in non radioactive product, it made possible to perform the above–mentioned characterizations.

Preparation of PAMAM G3.0 dendrimers encapsulated non radioactive Au nanoparticles was initiated by preparation of $HAuCl_4$ solution which was done by dissolving the gold foils in aqua regia. The nitroso was eliminated from gold solution by heating the gold solution untill dried. Au^{3+} solution formed a stable complex solution $[AuCl_4]^-$. Finally it was reconstituted in 0.01 M HCl to give 0.002 M $HAuCl_4$ solution. Absorbance of the prepared $HAuCl_4$ solution was then measured with UV-Vis spectrophotometer. The UV-Vis spectra of $HAuCl_4$ solution from Au foil in Fig. 2 showed the same absorption peaks as peak absorption of standard $HAuCl_4$ solution at λ 312 nm. This proved that preparation of $HAuCl_4$ solution from Au foil in this study had been successful.

Ezumi *et al.* [15] studied the preparation of gold-dendrimer nanocomposite by reduction of $[AuCl_4]^-$ with sodium borohydride ($NaBH_4$) in the presence of dendrimer. In this study however, the synthesis method was performed with a bottom-up method, in which a solution $HAuCl_4$ was reacted with PAMAM G3.0 dendrimer which was then followed by addition of $NaBH_4$ as a reductor. The color change after addition of $NaBH_4$ was observed indicating the formation of Au (0) nanoparticles. The yellow color of $HAuCl_4$ turned into ruby colour as a result of surface plasmon resonance (SPR) [20,21]. The spectra of F1, F2, and F3 shown in Fig. 3 did not give a peak absorption of $HAuCl_4$ at λ 312 nm which indicated that Au (III) was succesfully reducted into Au (0) nanoparticles.

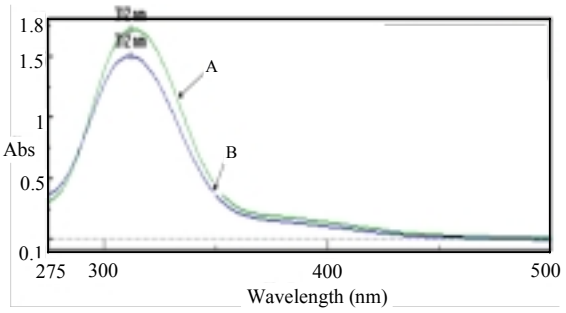

Fig. 2. Spectra of standard $HAuCl_4$ in HCl 0.01 M (A) and $HAuCl_4$ from Au foil (B).

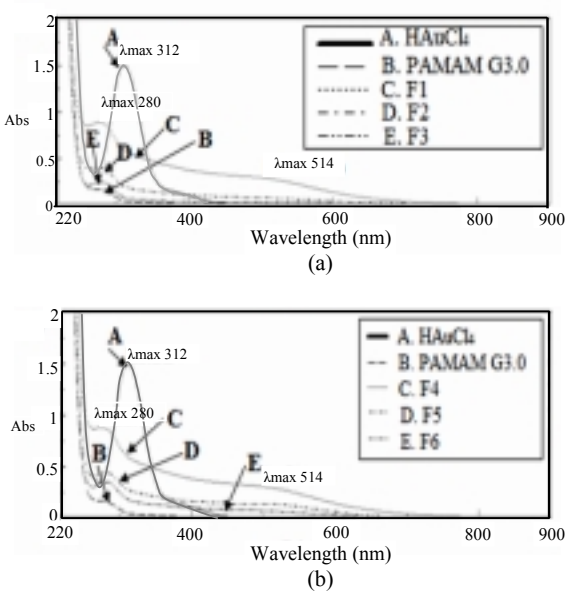

Fig. 3. Spectra of $HAuCl_4$, PAMAM G3.0, F1, F2, and F3 (a). Spectra of $HAuCl_4$, PAMAM G3.0, F4, F5, and F6 (b).

It can be seen in Fig. 3 that F1, F2, and F3 spectra gave the same pattern with the absorption peak at λ 280 nm and λ 514 nm. SPB at around λ 514 nm also indicated the formation of Au (0) nanoparticles that was larger than 2 nm in diameter and formed outside the cavity of PAMAM G3.0 dendrimers [8]. These spectra revealed that the increasing of molar ratios of PAMAM G3.0 dendrimers towards $HAuCl_4$ in F1, F2, and F3 reaction mixtures (0.7, 1.4 and 2.8) increased the absorption at the λ 280 nm and λ 514 nm. Similar pattern was also obtained in F4, F5, and F6 (molar ratios of PAMAM G3.0 dendrimers towards $HAuCl_4$ were 1.4, 2.8 and 5.6 respectively). Synthesis of PAMAM G3.0 dendrimers encapsulated Au nanoparticles according to Khan *et al*. [5] consisted of three-step reaction: 1) formation coordination complex between free electron of interior tertiary amine PAMAM dendrimer with $[AuCl_4]^-$ ion; 2) decomposition of the complex; 3) reduction of Au^{3+} to Au (0) nanoparticles with the addition of $NaBH_4$. In this study PAMAM G3.0 dendrimer was used as a template to control the growth of Au nanoparticles. Therefore, Au (0) nanoparticles was formed and trapped in the cavity of PAMAM G3.0 dendrimer.

The size of PAMAM is indicated by its generation. Higher generation of PAMAM has bigger diameter size compared to that of lower generation [9]. PAMAM G5.0 which has a diameter of approximately 50 ^{0}A or 5 nm will have the ability to encapsulate Au nanoparticles measuring of 1-3 nm in its cavity [20]. Therefore, PAMAM G3.0 which has a diameter of 3.6 nm is expected to encapsulate Au nanoparticles that are smaller than 2.0 nm in its cavity. In order to remove Au nanoparticles that are larger than 2.0 nm the raw product of PAMAM G3.0 dendrimers encapsulated Au nanoparticles needs to be purified. by SE-Chromatography (SEC) by a using PD-10 column is aimed to eliminate the Au nanoparticles which have diameter larger than 2 nm.

In this research purification of PAMAM G3.0 dendrimers encapsulated Au nanoparticles was performed by SEC Chromatography a using PD-10 column. The raw product of PAMAM G3.0 dendrimers encapsulated Au nanoparticles PD-10 column pre blocked with BSA and pre-equibrated with 0.01 M PBS pH 7.5. About 10 fractions (1 mL/ fraction) were collected and then analyzed with UV-Vis spectroscopy. In the SEC method, compounds with larger molecular weight will be eluted first, while the compounds with smaller molecular weight will eluted later. The results of UV-Vis spectroscopy analysis showed that fractions 4 indicated the presence of PAMAM G3.0 dendrimers encapsulated Au nanoparticles. This fraction did not show any absorption at 514 nm which was a characteristic of Au (0) nanoparticles with diameter larger than 2 nm and is not encapsulated by PAMAM G3.0 dendrimers. The existence of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticles in this fractions was also confirmed by colorimetric test. This fraction and PAMAM G3.0 solution gave a same color, blue, when treated with BioRad dye solution.

In order to determine the morphology of PAMAM G3.0 dendrimers encapsulated Au nanoparticles which were produced from reaction mixture of F1, F2, F3, F4, F5, and F6. The purified PAMAM G3.0 dendrimers encapsulated Au nanoparticles were tested by using a TEM and their images were showed in Fig. 4. As it can be seen in Fig. 4 that the morphology of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticle generated from F1 and F4 solutions were less uniform and spheris Au (0). The morphology of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticle generated from F2, F3, F5, and F6 solutions however were a uniform and spheris. This is influenced by the $HAuCl_4$ concentration, where a smaller concentration was used the more uniform morphology was obtained.

The average diameter of Au (0) nanoparticles and the entrapment eficiency were analyzed using PSA and AAS, respectively. The average diameter of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticles generated from F1, F2, F3, F4, F5, and F6 solutions were 3.299 nm, 201.2 nm, 0.7141 nm, 1.808 nm, 1.743 nm, and 4.199 nm,

respectively. The entrapment efficiency of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticle generated from F1, F2, F3, F4, F5, and F6 solutions which were measure by AAS were 1.24, 4.45, 3.3, 0.41, 26.34, and 8.26% respectively. It can be seen that the increasing molar ratios of PAMAM G3.0 dendrimers towards $HAuCl_4$ decreased the average diameter of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticles obtained except for F2 and F6 which had the average diameter of 201.2 nm and 4.199 nm, respectively. Anomaly was found for PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticles generated from F2 solution. This was suspected to be caused by several factors, among others, the occurrence of Au nanoparticles agglomeration, microbial contamination or the dust that interfere with the analysis process [22]. Therefore, it is important to strengthen the analytical results of this PSA with other nanoparticles characterization techniques such as TEM.

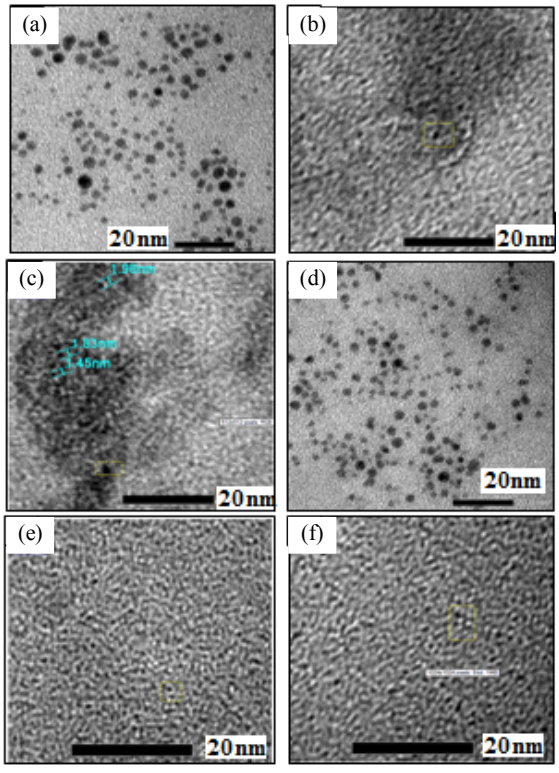

Fig. 4. The morphology images of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticle generated from (a) F1, (b), F2, (c) F3, (d) F4, (e) F5 and (f) F6 solutions.

It has been mentioned before that nanoparticles with the average size larger than 2.0 nm were not encapsulated by PAMAM G3.0. There are several possible interactions between Au nanoparticles with PAMAM G3.0, that is through its interaction with the tertiary amine in the interior or cavity of PAMAM G3.0. Another is the interaction of Au nanoparticles with the primary amine surface of the PAMAM G3.0 or exterior amine functional groups [20]. Characterization by using particle size analyzer revealed that Au (0) nanoparticles derived from F1, F2, and F6 solutions were formed in the cavity of PAMAM G3.0 dendrimers, but Au (0) nanoparticles derived from F3, F4, and F5 solutions showed that formation of Au (0) nanoparticles were outside the cavity of PAMAM G3.0 dendrimers. The analysis of Au (0) nanoparticles interaction with PAMAM G3.0 dendrimers still need a further study.

Based on the above results, purification carried out by PD-10 column was a quite effective method for separation of PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticles from Au nanoparticles that are smaller than 2.0 nm as it was supported and confirmed by TEM characterization results. It could also be seen that the molar ratios of PAMAM G3.0 dendrimers to Au^{3+} influenced not only the amount of Au (0) nanoparticles that entrapped in the cavity of PAMAM G3.0 dendrimers but also the morphology and the average diameter. The preliminary study also showed that the best formulation for preparation PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticles was F5 solution where molar ratio of PAMAM G3.0 dendrimers towards $HAuCl_4$ was 2.8, because this formulation was able to produce PAMAM G3.0 dendrimers encapsulated Au (0) nanoparticles with the adsorption efficiency value of 26.34%, the size of Au nanoparticles that are less than 2 nm, and a spherical morphology and uniform. It was therefore this formulation was then used for preparation of PAMAM G3.0 dendrimers encapsulated ^{198}Au (0) nanoparticles.

Synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles "hot synthesis".

Synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles can be done in two ways, first by irradiating the Au nanoparticles-PAMAM G3.0, and second by using the solution of $H^{198}AuCl_4$. This study used the later method, as there are some disadvantages when irradiating the PAMAM G3.0 dendrimers encapsulated Au nanoparticles. The disadvantages that had reported were the formation of radical product, expansion of molecules, etc [5]. Preparation of $H^{198}AuCl_4$ solution was carried out by irradiating Au foil (^{197}Au) in the GA Siwabesy Multi Purposes Reactor with neutron capture reaction, with neutron flux of

1.2 x 10^{13} n/cm^2/s for five hours. Isotope ^{197}Au is a stable isotope with a large abundance in nature (-99.99%). If ^{197}Au captures neutron, it will produce ^{198}Au radioisotope [23]. Neutron capture reaction (n, γ) can be performed due to the large cross section reaction of ^{197}Au, 98.65 barn (1 barn = 10^{-24} cm^2) [23].

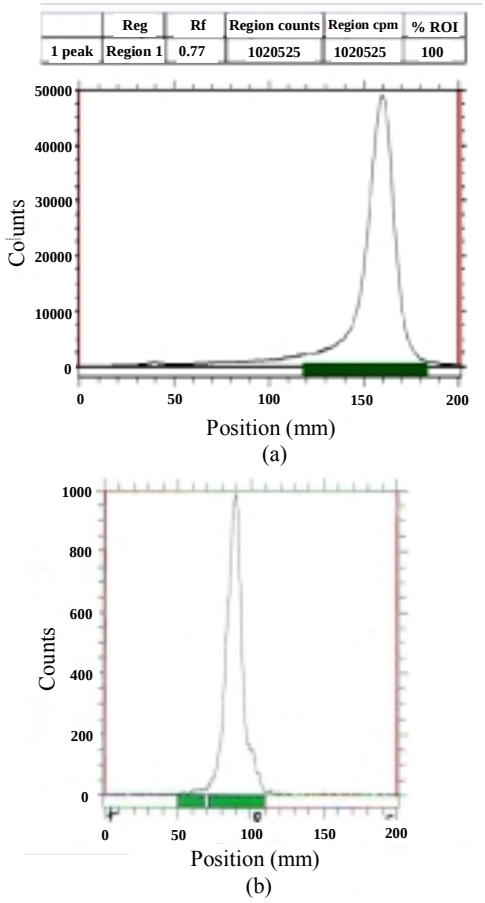

Fig. 5. Radiochromatogram of $H^{198}AuCl_4$ solution (a) analysed with TLC and (b) analyzed with electrophoresis.

Preparation of $[^{198}AuCl_4]^-$ from Au foil resulted in a high purity of ^{198}Au radionuclide. The preparation of $[^{198}AuCl_4]^-$ was carried out in the same manner as the preparation of the non-radioactive $[AuCl_4]^-$ solution. ^{198}Au foil was dissolved in aqua regia and the $[^{198}AuCl_4]^-$ solution produced was then analyzed by a dose calibrator to measure it radioactivity. In order to determine its chromatographic profile $[^{198}AuCl_4]^-$ solution was also analyzed by a thin layer chromatography (TLC) as well as electrophoresis. The TLC of $[^{198}AuCl_4]^-$ solution which was carried on a Whatman paper No. 1 and 70% of methanol as stationary and mobile phase, respectively gave a chromatogram with Rf of 0.77, shown in Fig. 5a. Analysis of $[^{198}AuCl_4]^-$ with electrophoresis by using a Whatman paper No. 1 and 0.01 M phosphate buffer pH 7.5 gave a chromatogram shown in Fig. 5b. This chromatogram indicated that $[^{198}AuCl_4]^-$ was the only species in the $H^{198}AuCl_4$ solution which was prepared from Au foil.

Synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles from $[^{198}AuCl_4]^-$ was conducted using F5 solution formula (molar ratio PAMAM G3.0 dendrimers to ^{198}Au/Au was 2.8) where radioactivity of $[^{198}AuCl_4]^-$ used was of 175.8 µCi. After reduction the raw product of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles was purified with SEC using a PD 10 column. Ten fractions (1 mL / fractions) were retrieved from this purification process.

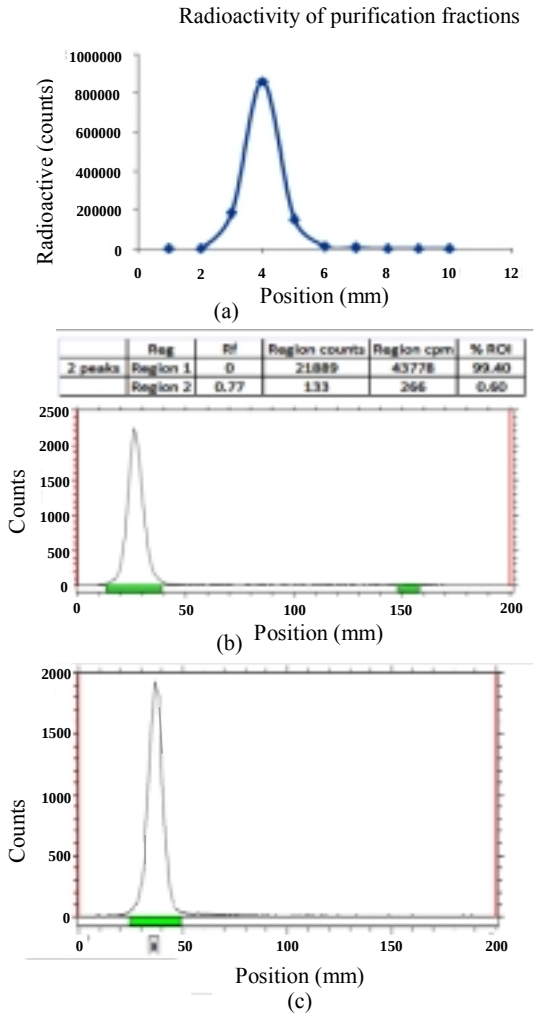

Fig. 6. (a) Radiochromatogram of purification using PD-10 column, (b) Radiochromatogram of fraction 4 analyzed with TLC, (c) Radiochromatogram of Fraction 4 analyzed with Electrophoresis.

Figure 6a shows the radiochromatogram of PAMAM G3.0 dendrimers encapsulated ^{198}Au

nanoparticles purification. It can be seen from this chromatogram having a peak at fraction 4. When aliquot of this fraction was tested with Bio-Rad dye solution, it gave a blue color which indicated the existence of PAMAM G3.0 dendrimers. This fraction was then further analyzed with TLC by using a Whatman paper No. 1 and 70% of methanol as stationary and mobile phase, respectively and electrophoresis by using Whatman paper No.1 and phosphate buffer 0.01 M pH 7.5. The chromatograms of fraction 4 which were generated from the above-mentioned analysis were shown in Fig. 6b (TLC) and 6c (Electrophoresis). It can be seen in Fig. 6b that Rf of fraction 4 was 0. This indicated there was no more $[^{198}AuCl_4]^-$ (Rf > 0.7) species in this fraction. It also can be seen in Fig. 6c that fraction 4 had a zero charged. Based the above data it could be proved that the species that was exist in fraction 4 was of PAMAM G3.0 dendrimers encapsulated Au nanoparticles and the radiochemical purity of PAMAM G3.0 dendrimers encapsulated Au nanoparticles was found to be 99.4% and zero charged.

CONCLUSION

Synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles was successfully performed by using a bottom-up method which was previously established by using non-radioactive Au. Synthesis of PAMAM G3.0 dendrimers encapsulated non-radioactive Au nanoparticles was carried out prior to the synthesis of PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles in order to be able to fully characterize the of PAMAM G3.0 dendrimers encapsulated Au nanoparticles produced. The PAMAM G3.0 dendrimers encapsulated non-radioactive Au nanoparticles was most effective when prepared from PAMAM G3.0 dendrimers and $HAuCl_4$ with molar ratio of 2.8. Purification with SEC method by using PD-10 was proved to be quite effective to purify PAMAM G3.0 dendrimers encapsulated non-radioactive Au nanoparticles from Au nanoparticles > 2 nm. Characterization by TEM, PSA and AAS revealed that PAMAM G3.0 dendrimers encapsulated non-radioactive Au nanoparticles produced were uniform and spheris which had a diameter < 2 nm and encapsulated in interior or cavity PAMAM G3.0. PAMAM G3.0 dendrimers encapsulated ^{198}Au nanoparticles which was prepared precisely in a similar manner with the preparation PAMAM G3.0 dendrimers encapsulated non-radioactive Au nanoparticles were found to have radiochemical purity of radiochemical purity of 99.4% and zero charge.

Future research to find out the stability of PAMAM G3.0 dendrimers encapsulated ^{198}Au *in vitro* and *in vivo* using tumor cell line and animal model, respectively, need to be carried out.

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