Iodogen Method on Iodine-131 (\(^{131}\text{I}\)) Radiolabelling of Silver Nanoparticle (AgNPs) as a New Agent of Molecular Imaging

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Abstract. The application of nanomaterials in the treatment of various types of diseases continues to increase, including the use of silver nanoparticles (AgNPs). However, there still limitation in terms of the research on labelling AgNPs using radioactive compound such as \(^{131}\text{I}\). The aim of this study is to carry out a method on \(^{131}\text{I}\) radiolabelling of AgNPs by using Iodogen as an iodination reagent. The radiolabelled \(^{131}\text{I}\)-AgNPs were then purified by using Sephadex-25 column chromatography with 0,05 M phosphate buffer solution as mobile phase for the first purification and HEPES solution for the second purification. The radiochemical purity of radiolabelled \(^{131}\text{I}\)-AgNPs was then determined by using autoradiography scanner. \(^{131}\text{I}\)-AgNPs with a purity 94,5±0,2121% were obtained after the purification. Stability test of the \(^{131}\text{I}\)-AgNPs was carried out by determining the radiochemical purity of the \(^{131}\text{I}\)-AgNPs on the first day until the fifth day of storage in the room temperature and refrigerator. The best stability of the \(^{131}\text{I}\)-AgNPs after purification resulted in radiochemical purity >90% until the fourth day and <90% on the fifth and subsequent days in both storages. This result shows that storage in the refrigerator can be a better choice rather than in the room temperature.

Keywords: Silver Nanoparticle, \(^{131}\text{I}\), \(^{131}\text{I}\)-AgNPs, Iodogen, Labelled Compound, Autoradiography Scanner

1. Introduction
The application of nanomaterials in both organic or inorganic for the treatment of various types of diseases continues to increase. One of the most popular inorganic nanomaterials is silver nanoparticle. Silver nanoparticles are famous for their ability as broad-spectrum antimicrobials and antiviral [1,2]. The study also found the application of silver nanoparticle as photosensitizers and/or radiosensitizers, anticancer therapeutic agents, wound dressing, textiles, and biomedical devices [2,3].

The study of radioactive Iodine has been done for a long time ago. \(^{131}\text{I}\) is one of iodine isotope that has 8 days half-life [4]. \(^{131}\text{I}\) can produce by using neutron activation of Tellurium-130 dioxide\(^{130}\text{TeO}_2\) [5-6]. \(^{131}\text{I}\) can be applied in both diagnostic and therapy since it can emit gamma and beta [7]. \(^{131}\text{I}\) has been widely used in medical application such as thyroid cancer staging, treatment of hyperthyroidism and differentiated thyroid cancer, treatment of metastatic tumours, neuroblastoma diagnosis dan therapy, etc [8-11]. Thus, \(^{131}\text{I}\) still become an important isotope in nuclear medicine.
Nowadays, the application of nanomaterials in radiopharmaceuticals has attracted huge attention. In 2010, a method on rapid Iodine-125 ($^{125}$I) radionuclide labeling of silver nanoparticles for in vivo SPECT has been published by Christina and Schnitzer [12]. Even though they have different energy and half life, $^{125}$I and $^{131}$I have similar chemical properties. So that, there is a possibility to label AgNPs with $^{131}$I. In terms of diagnosis and therapy, both of $^{125}$I and $^{131}$I can be used. The production of $^{125}$I isotope is using natural or enriched Xenon-124 gas as target material [13,14]. Thus, this process need an adequate facility. Due to this requirement, there are a limitations in production process of $^{125}$I specially in Indonesia. In different with $^{125}$I, $^{131}$I has a high production capacity in Indonesia. PTRR-BATAN can produce up to 9 Ci of $^{131}$I for each batch production. So that, it is important to carry out a method on AgNPs labelled $^{131}$I as new agent in molecular imaging through radioiodination to support nuclear medicine application in Indonesia. Radioiodination is a molecule chemically modifying process to contain radioactive iodine. Radioiodination can be done by using directly or indirectly labeling. There many popular reagents indirect labeling such as chloramine-T, iodo-beads, iodogen, lactoperoxidase-catalyzed iodination, etc [15,16]. Iodogen is 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril, an N-haloamine derivative that similar to Iodo-beads in term of oxidizing properties. Radioiodination can be stopped easily by removing the aqueous phase in iodogen reaction. Iodogen is being used frequently to prepare any kind of radiolabeled compound such as peptides and probes [15].

In this research, a method on $^{131}$I radiolabelling of silver nanoparticles by using iodogen as the oxidative agent will be done. The stability test of synthesis of $^{131}$I-AgNPs will be carried out in the room temperature and refrigerator storage.

2. Experimental Method

2.1 Materials
This research was used several materials such as Silver nitrate, sodium chloride, universal pH indicator that were purchased from Merck, while Sodium borohydride, phosphate buffer, polyvinylpyrrolidone (PVP) and methanol were bought from Sigma Aldrich, and Iodogen were purchased from Pierce. Silver nitrate was used as the main material for the synthesis of the silver nanoparticles, sodium chloride and PVP as stabilizer for the silver nanoparticles [15], phosphate buffer as pH adjusting agent, sodium borohydride as reducing agent, methanol as mobile phase for radiochemical purity test of $^{131}$I-AgNPs, and iodogen as the iodination reagent. The equipment was used include glass equipment, magnetic stirrer, micropipette, disposable cuvette, SPHADEX-25 column and vial. The instruments were used consist of an analytical balance (Acculab), dose calibrator (Atomlabplus), UV-Vis Spectrophotometer (Jasco32), Particle Size Analyzer (PSA), Transmission Electron Microscopy (TEM) in LIPI Physics Research Center, and Autoradiography Scanner (Cyclone-Plus).

2.2 Synthesis and Characterization of AgNPs
AgNPs were synthesized by reduction method by using sodium borohydride as the reducing agent as explained by Umi et al. [17]. The characterization of AgNPs was determined by using transmission electron microscopy (TEM), UV-Vis spectrophotometer, and particle size analyzer (PSA).

2.3 Preparation of 10 mM HEPES solution
0.598 g of powdered HEPES was dissolved by using 250 mL of demineralized water and were stirred until homogeneous. The HEPES solution pH was then adjusted until 7 by using HNO3 or NaOH.

2.4 Preparation of Iodogen for Iodination
1 mg of iodogen were dissolved by using 1 mL of chloroform.100 µL of this solution were prepared into 2 glass vessels respectively. The first vessel was used for labeling of AgNPs by using $^{131}$I and the other for the control. The solvent was evaporated slowly by using a stream of nitrogen gas. The vessels were then sealed until used.
2.5 Labelling AgNPs by Using $^{131}$I
1 mL of AgNPs were added into the first vessels that had been prepared before. 20 µL of 0.5 M phosphate buffer pH 7 solution was added and followed by adding ~1mCi of Na$^{131}$I. The solution was vortex and left for 1 minute. 200 µL of 0.05 M phosphate buffer pH 7.4 solution were then added. The radiochemical purity test was then determined by using thin paper chromatography which Whatman paper 1 as stationary phase and methanol 75% as mobile phase.

2.6 Preparation of Control
20 µL of 0.5 M phosphate buffer pH 7 solution was added into the second vessels that had been prepared before and followed by adding ~1mCi of Na$^{131}$I. The solution was vortex and left for 1 minute. 200 µL of 0.05 M phosphate buffer pH 7.4 solution were then added. The radiochemical purity test was then determined by using thin paper chromatography which Whatman paper 1 as stationary phase and methanol 75% as mobile phase.

2.7 Purification 1
The Sephadex G-25 column was prepared and installed on a stative, then the tip was cut out until all the water inside the column discharged. The column was then washed out by using a 0.05 M phosphate buffer pH 7.4 solution until the filtrate that coming out of the column has pH 7.4. The $^{131}$I-AgNPs solution was poured into the column and was eluted by using a 0.05 M phosphate buffer pH 7.4 solution. The filtrate was then collected 500 µL for each fraction and the radioactivity was measured respectively by using dose calibrator. The radiochemical purity of the fraction with the highest radioactivity was then determined by using paper chromatography method with Whatman paper 1 as stationary phase and methanol 75% as mobile phase.

2.8 Purification 2
The Sephadex G-25 column was put on a stative, and all of the solutions inside was discharged. The column was then conditioned by using 10 mM HEPES pH 7 solution until the filtrate that removed out of the column has pH 7. The $^{131}$I-AgNPs solution was set into the column and was eluted by using 10 mM HEPES pH 7 solution. The filtrate was then collected 500 µL for each fraction and the radioactivity was measured respectively by using dose calibrator. The radiochemical purity of the fraction with the highest radioactivity was then determined by using paper chromatography method with Whatman paper 1 as stationary phase and methanol 75% as mobile phase.

2.9 Stability Test of $^{131}$I-AgNPs
The stability test of $^{131}$I-AgNPs was done by the determination of the radiochemical purity of 3 fractions of $^{131}$I-AgNPs with the highest activity and radiochemical purity which were stored in the room temperature and refrigerator. Each fraction were spotted in the Whatman paper as stationary phase and methanol 75% as mobile phase and the radioactivity was measured by using autoradiography scanner.

3. Results and Discussion
Physical examination of synthesis of AgNPs is a deep yellow-solution, clear, without precipitation. Figure 1 And 2 show the characterization of AgNPs by using Particle Size Analyzer (PSA) and Transmission Electron Microscopy (TEM), respectively. PSA result shows that the average distribution result of AgNPs is 14.5 nm.
Figure 1. Characterization of AgNPs by using PSA.

Figure 2. Characterization of AgNPs by using TEM.

The labelling process of AgNPs by using $^{131}$I was proceeded by the iodination process. In this research, iodogen was used as an oxidizing agent. Radiiodine may interact with AgNPs by absorption mechanism. This labelling method has resulted in $^{131}$I-AgNPs with pH 7 and radiochemical purity (RCP) as shown in Figure 3-5 and Table 1.

Table 1. Radiochemical Purity of $^{131}$I-AgNPs.

| Sample  | Activity (µCi) | RCP (%)       | Retention Factor (cm) | Appearance     |
|---------|----------------|---------------|-----------------------|----------------|
| Bulk $^{131}$I | 1,551          | 99.2          | 0.75                  | Clear solution |
| $^{131}$I-Iodogen     | 1,489          | 99.2 ± 0.2828 | 0.69                  | Clear solution |
| $^{131}$I-AgNPs       | 1,131          | 80.4 ± 0.2121 | 0.29                  | Yellow-clear solution |

Radiochemical purity resulted in the labelling of AgNPs by using $^{131}$I with iodogen as the oxidizing agent was 80.4%. Since the radiochemical purity of $^{131}$I-AgNPs was still low, $^{131}$I-AgNPs were then purified by using Sephadex-25 column with phosphate buffer 0.05 M as mobile phase. Each 500 µL of the filtrate were collected and the radioactivity were measured by using Dose Calibrator as shown in Figure 6.
Figure 6. The Elution Profile of Purification 1 of the $^{131}$I-AgNPs with phosphate buffer 0.05 M as mobile phase.

The purification resulted in three fractions with the highest activity were fraction 6, 7 and 8. The radiochemical purity test of these fractions was then determined and the result as shown in Table 2 and Figure 7-9.

Table 2. Radiochemical Purity of $^{131}$I-AgNPs after purification 1.

| Fraction | RCP (%)       | Retention Factor (cm) | Appearance          |
|----------|---------------|-----------------------|---------------------|
| Fraction 6 | 59.5 ± 3.1113 | 0.29                  | Yellow-clear solution |
| Fraction 7 | 48.8 ± 0.2828 | 0.29                  | Yellow-clear solution |
| Fraction 8 | 60.7 ± 0.0707 | 0.29                  | Yellow-clear solution |

As shown in Table 2, the radiochemical purity of $^{131}$I-AgNPs was decreased to less than 65%. As a result, the three fractions with the highest activity in purification 1 were mixed and purified with HEPES 10 mM pH 7 as mobile phase. Each 1 mL of filtrate was collected and the radioactivity was measured by using Dose Calibrator as shown in Figure 10.
Figure 10. The Elution Profile of Purification 2 of the $^{131}$I-AgNPs with HEPES 10 mM pH 7 as mobile phase.

The radiochemical purity of the fractions 5, 6, and 7 as the fraction with the highest radioactivity of more than 90% was then determined by using Autoradiography scanner. The result shows that the fraction 6 has the highest radiochemical purity of 94.6% compared to the fraction 5 and 7 as shown in Table 3.

Table 3. Radiochemical Purity of $^{131}$I-AgNPs after purification 2

| Fraction | RCP (%)   | Retention Factor (cm) | Appearance       |
|----------|-----------|-----------------------|------------------|
| Fraction 5 | 90.4 ± 0.3536 | 0.18                  | Yellow-clear solution |
| Fraction 6 | 94.5 ± 0.2121  | 0.18                  | Yellow-clear solution |
| Fraction 7 | 91.2 ± 0.8485  | 0.19                  | Yellow-clear solution |

Figure 11. The Autoradiogram of Radiochemical Purity of $^{131}$I-AgNPs fraction 5.

Figure 12. The Autoradiogram of Radiochemical Purity of $^{131}$I-AgNPs fraction 6.

Figure 13. The Autoradiogram of Radiochemical Purity of $^{131}$I-AgNPs fraction 7.

Purification 2 shows the result that fraction 6 has the highest activity of 178.7 µCi and highest radiochemical purity of 94.6% compared to the fraction 5 and 7. These fractions were then stored in the room temperature and refrigerator to determine the stability of the $^{131}$I-AgNPs after labelling and purification process.
Figure 14. Comparison of Radiochemical Purity of Fraction 6 of $^{131}$I-AgNPs.

Figure 15. Comparison of Radiochemical Purity of Fraction 5 of $^{131}$I-AgNPs.

Figure 16. Comparison of Radiochemical Purity of Fraction 7 of $^{131}$I-AgNPs.

Figure 14-16 shown the comparison of radiochemical purity of fraction 5-7 of $^{131}$I-AgNPs after purification 2. All of the three fractions have radiochemical purity more than 90% until the 3rd day after purification in both storages. However, in the 4th day after purification, only fraction 6 that shown $^{131}$I-AgNPs with radiochemical purity more than 90% in both storages. In the 5th day after purification, all of the three fractions have radiochemical purity less than 90%. All of the three fractions stored in room temperature show radiochemical purity less than 50%, while the fractions that stored in the refrigerator show radiochemical purity more than 50% with fraction 6 still have radiochemical purity of 80%. Only fraction 6 that stored in a refrigerator that shows radiochemical purity up to 80% while the others show less. This result shows that storage in the refrigerator can be a better choice rather than in the room temperature.
4. Conclusion

Iodogen as the oxidizing agent on $^{131}$I radiolabelling of AgNPs resulted in $^{131}$I-AgNPs with radiochemical purity $80.4 \pm 0.2121\%$ and $94.5 \pm 0.2121\%$ after purification. Stability test shows that the best $^{131}$I-AgNPs has radiochemical purity more than $90\%$ in both storages (room temperature and refrigerator) until the $4^{th}$ day after purification. Only $^{131}$I-AgNPs with the highest activity that stored in a refrigerator that shows radiochemical purity up to $80\%$ while the others show less. $^{131}$I-AgNPs can be used until the $4^{th}$ day after purification as long as the radiochemical purity still more than $90\%$. Storage in the refrigerator can be a better choice rather than in the room temperature.

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