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Preparation of ^{99m}Tc-Kanamycin using a Direct Labeling Method

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ABSTRACT

Infectious diseases are still the leading cause of death in the world. The accurate technique for early detection and determination of the exact location of infection in the body is still needed. Nuclear techniques are capable for this purpose while other techniques such as MRI, USG and CT-SCAN sometimes cannot be applied. ^{99m}Tc-kanamycin radiopharmaceutical is complex of kanamycin and technetium-99m radionuclide, was used for bacterial detection of infection. The labeling studies of ^{99m}Tc-kanamycin has been carried out by the indirect labeling method using pyrophosphate as a co-ligand with the results of labeling efficiency above 95%. However, the presence of radiochemical impurities in the form of ^{99m}Tc-pyrophosphate in the indirect labeling have to be considered which may interfere the imaging result. This study aimed to determine the optimum labeling conditions of ^{99m}Tc-kanamycin by direct labeling method. Kanamycin was successfully labeled with technetium-99m through direct labeling method. The labeling efficiency was determined by ascending paper chromatography using Whatman 3 paper as the stationary phase, and acetone as the mobile phase to separate the radiochemical impurities in the form of ^{99m}Tc-pertechnetate. While impurities in the form of ^{99m}Tc-reduced were separated using the stationary phase ITLC-SG and 0.5 N NaOH as mobile phase. The experiment result showed that the optimum labeling conditions obtained by using 5 mg kanamycin, 30 µg $SnCl_2.2H_2O$, and pH of labeling was 9. The incubation time of labeling was 30 min at room temperature, provided labeling efficiency of 92.31 ± 1.74 %. The successful of kanamycin labeling with high efficiency makes ^{99m}Tc-kanamycin can potentially be used as a radiopharmaceutical for the early detection of infectious diseases.

INTRODUCTION

Infection is the invasion and proliferation of microorganisms in body tissue. Clinically, an infection may not be apparent; however, local cellular injury may arise instead as a result of metabolism competition, toxins, intracellular replication, or antigen-antibody response [1]. Infectious diseases are one of the global health problems both in developed countries and in developing countries such as Indonesia. One disease which is caused by infections, or infectious disease, namely the tuberculosis (TB), still puts a strain on public health, being second only to HIV/AIDS in causing high mortality rates [2]. It has been reported that in 2013, there were about 9 million new cases and 1.5 million deaths due to TB [2-4] with about two billion people latently infected [5]. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged 15 to 44 [2]. Those TB deaths occur because of late identifications of main infections which occur in very deep parts of the body (deep-seated infection). The preferential detection of infection from sterile inflammations is one of the most difficult problems in medicine [6]. The available imaging techniques such as Plain Radiography, Ultrasonography, Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) have high sensitivity but are not specific for infection especially in early phases,

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when anatomical structures have not been distorted [6]. Early detection and determination of the exact and accurate location of the infection are indispensable. Here, nuclear techniques could complement the shortcomings of the other techniques.

The latest research activities in the field use ^{68}Ga-labeled radiopharmaceuticals with ^{68}Ga or ^{18}FDG and utilize PET/CT facilities for detecting infection [7-12]. A novel approach using radioactive-labelled antibiotics or antimicrobial peptides labelled with radionuclides, or radiolabeled antibiotics or antimicrobial peptides, could be a solution to distinguishing between infective and non-infective inflammations [1,3,14-21]. Researchers at PSTNT-BATAN have successfully developed two kinds of radiolabeled antibiotic, namely ^{99m}Tc-ciprofloxacin [22] and ^{99m}Tc-ethambutol [23]. The use of infecton (^{99m}Tc-ciprofloxacin) is a sensitive technique that aids in the earlier detection and treatment of a wide variety of deep-seated bacterial infections. The ability to localize infective foci accurately is also important for surgical intervention, such as drainage of abscesses. In addition, serial imaging with infecton is probably useful in monitoring clinical response and optimizing the duration of antimicrobials [24]. ^{99m}Tc-ethambutol is a specific and sensitive radiopharmaceutical to detect and determine the location of mycobacterial TB at an early stage in the anatomy of the body. The mycobacterial lesion uptake study in humans suggested that ^{99m}Tc-etambutol is specific and sensitive radiopharmaceutical for resistant tubercular lesion detection and localization [25].

The *Staphylococcus aureus* (*S. aureus*) is one the microorganisms most frequently involved in the pathogenesis of infection in clinical practice [6]. Kanamycin (Fig. 1) is an antibiotic belonging to the aminoglycosides that act by binding to the bacterial ribosome, thereby inhibiting protein synthesis and generating errors in the translation of the genetic code [26]. Its nature as a broad-spectrum antibiotic allows it to bind to Gram-negative and Gram-positive bacteria. It was first discovered in Japan in 1957 by Umezawa et al., is obtained from the culture filtrate of *Streptomyces kanamyceticus*. Kanamycin is used to treat infections when penicillin or other less toxic drugs cannot be used. Infections treated include: bone-, respiratory-tract-, skin-, soft-tissue-, and abdominal infections, complicated urinary-tract infections; endocarditis; septicemia; and enterococcal infections [27]. Based on the structure-activity relationships study of the aminoglycoside antibiotics conducted by Benveniste and Davies or S. Salian *et al*., the activity of this class of antibiotics is related to the position of hydroxyl groups and amines as substituents on R and R' (Fig 1). Kanamycin B and C are derivatives of kanamycin A. From in-vitro inhibition test of the antibiotic kanamycin of phage R17 RNA, it was found that kanamycin B has a stronger activity than kanamycin A, while kanamycin C has the weakest activity compared to both kanamycin A and B [28-29]. More researches have been devoted to chemical modification of aminoglycoside antibiotics such as kanamycin with the goal of increasing antibacterial activities or new activities as antibacterial [30-33].

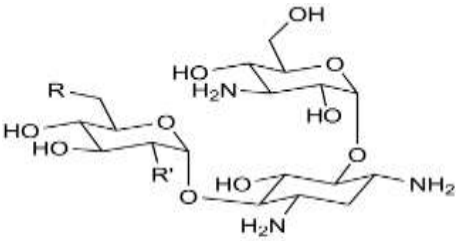


Fig. 1. Chemical structure of kanamycin.

^{99m}Tc-kanamycin labeling studies have been carried out in the previous research by the indirect method using pyrophosphate as a co-ligand with the result of labeling efficiency of above 95% [34-36]. However, the presence of radiochemical impurities in the form of ^{99m}Tc-pyrophosphate in this indirect labelling method may interfere with the imaging results. The accumulation of ^{99m}Tc-pyrophosphate in the bone made it difficult to distinguish between an infection of the bone and the uptake of ^{99m}Tc-pyrophosphate. Therefore, this study was conducted to label the antibiotic kanamycin with radionuclide ^{99m}Tc by the direct method. In addition, as the strong and broad-spectrum antibiotic kanamycin is expected to be able to detect the presence of infection in the human body, so ^{99m}Tc-kanamycin can be used as a radiopharmaceutical for detecting infections.

EXPERIMENTAL METHODS

The materials used to carry out this research were kanamycin sulfate (Meiji), tin(II) chloride/$SnCl_2$ (Sigma-Aldrich), acetone (E. Merck), sodium hydroxide (E. Merck), physiological sodium chloride (IPHA), sterile aquabidest (double-distilled water) (IPHA), pH indicator (E. Merck), Whatman no. 3 paper chromatography, and ITLC-SG (Agilent).

The equipment used in this experiment consists of dose calibrator (Victoreen), vortex mixer, single channel analyzer (Ortec), and paper chromatography apparatus.

Labeling kanamycin with direct method

The direct labeling of kanamycin with ^{99m}Tc was performed using $SnCl_2.2H_2O$ as reducing agent. The optimum conditions were obtained by varying the various parameters such as amount of reducing agents, amount of kanamycin, pH level, and incubation time. The labeling process was carried out by adding a solution of $SnCl_2$ (1 mg/mL in water) into a solution of kanamycin in water; the pH of the solution was adjusted by adding 0.1 N NaOH / HCl. Then, a saline solution of $^{99m}TcO_4^-$ with an activity of 2-5 mCi was injected into the vial and its volume was adjusted to 2 mL. All experiments were carried out in a volume of 2 mL and with incubation at room temperature.

Determination of labeling efficiency of ^{99m}Tc-kanamycin

The determination of labeling efficiency was done simultaneously with the determination of the radiochemical purity of ^{99m}Tc-kanamycin using ascending paper chromatography method using Whatman no. 3 paper (10 × 1 cm) as the stationary phase and acetone as the mobile phase to separate the impurities of ^{99m}Tc-pertechnetat ($^{99m}TcO_4^-$) form at R_f = 1.0. Meanwhile, to separate the impurity of ^{99m}Tc-reduced ($^{99m}TcO_2$), ITLC-SG (10 × 1 cm) was used as the stationary phase and 0.5 N NaOH as a mobile phase where R_f of $^{99m}TcO_2$ = 0.0. The chromatograms were dried in oven at 80 ^oC for five minutes, and then every 1 cm piece of paper was cut and measured using single-channel gamma counter with detector NaI(Tl).

Optimization of reducing agent $SnCl_2.2H_2O$ amount

The determination of the optimal amount of reducing agent was performed as follows: To a series of vials containing kanamycin solution (6 mg/mL), solutions of $SnCl_2$ (1 mg/mL) of varying amounts (20, 35, 30, and 35 µL) were added. The pH of the mixture was adjusted to 6-7 by adding a 0.5 N NaOH solution, and then a solution $Na^{99m}TcO_4$ with an activity of 2-5 mCi was added. The final volume was then adjusted to 2 mL. The mixture was shaken with a vortex mixer until homogenous and was then incubated at room temperature for 30 minutes. The optimum amount of reducing agent was determined from the labeling efficiency of ^{99m}Tc kanamycin using paper chromatographic method as described above.

Optimization of kanamycin amount

Into each vial containing varying concentrations of kanamycin (3, 4, 5, 6, and 7 mg/mL) was added 30 µL solution of $SnCl_2$ (1 mg/mL). The solution's pH was adjusted to 6-7 by the addition of 0.5 N NaOH solution. After the addition of all reagents, then 2-5 mCi of $^{99m}TcO_4^-$ in saline was injected into each vial so that the final volume in all experiments was 2 mL. The mixture was shaken with a vortex mixer and incubated at room temperature for 30 minutes. The optimum amount of kanamycin was determined from the labeling efficiency of ^{99m}Tc kanamycin using paper chromatographic method.

Optimization of pH

Into five vials, each containing 1 mL kanamycin (5 mg/mL), 30 µL of $SnCl_2$ solution (1 mg/mL) was added. The mixture was shaken gently until homogeneous. The pH of the solutions in those vials was adjusted to 4, 5, 6, 7, 8 and 9, respectively, by adding 0.5 N NaOH, and then to the mixture was added a solution of $Na^{99m}TcO_4$ with activity of 2-5 mCi, and the final volume of the mixture was adjusted to 2 mL. The mixtures were shaken gently until homogeneous and incubated at room temperature for 30 minutes. The optimum pH was determined from the labeling efficiency of ^{99m}Tc kanamycin using a paper chromatographic method.

Optimization of incubation time

Labeling was done by adding 30 µL of $SnCl_2$ solution (1 mg/mL) into a vial containing 5 mg of kanamycin that was dissolved in 1 mL of aquabidest and stirring gently until homogeneous. The mixture was incubated for 15 minutes at room temperature and then the pH was adjusted to 9 by addition of 0.5 N NaOH. After the intended pH of the mixture was reached, then 2-5 mCi of $Na^{99m}TcO_4$ solution was added and the final volume of the solution was adjusted to 2 mL. The mixture was stirred gently until homogeneous and incubated at room temperature for varying periods of 15, 20, 25, and 30 minutes. The optimum incubation time is

the time that gives a high labeling efficiency of ^{99m}Tc-kanamycin. The labeling efficiency was determined when the intended incubation interval was reached; the labeling efficiency was determined using a paper chromatography method.

RESULTS AND DISCUSSION

A radiopharmaceutical could be defined as a chemical substance that contains one or more radioactive atoms within its structure and is suitable for administration to humans for diagnosis or treatment of a disease [37]. The ^{99m}Tc-radiopharmaceuticals which are used for clinical purpose in nuclear medicine have been developed from the mid-1960s until now. ^{99m}Tc-radiopharmaceuticals are metal complexes, prepared by reducing ^{99m}Tc-pertechnetate to a lower oxidation state. The coordination complexes of technetium are formed of bonds between technetium acting as Lewis acid, and atoms or functional groups, which act as Lewis bases [38]. In preparing a new radiopharmaceuticals, there are several factor to be considered, one of them is the compatibility of the isotope will be incorporated into the molecule to be labeled [39]. Kanamycin has several functional groups such as $–NH_2$, –OH, and –O– to form bonds with ^{99m}Tc. Although the details of the chemistry of formation and molecular structure are unknown, ^{99m}Tc-Kanamycin is assumed to be a chelate complex with one or more Kanamycin ligands attached to reduced ^{99m}Tc [27]. Using chemical software (Chem Bio Ultra 11.0) by considering electronegativity of functional groups that have electrons donor and steric hindrance, we assumed the chelate complex of ^{99m}Tc-kanamycin are oxo-technetium complexes that shown in Fig. 2. The statement was based on the quite large number of radiopharmaceutical which have a +5 oxidation state with TcO^{3+} or TcO_2^+ core. So, the possible core complexes of ^{99m}Tc-kanamycin are $TcO(N)_4$ or $TcO_2(N)_4$ core complexes. Based on Technetium Chemistry and Radiopharmaceutical Applications book [40], the reaction of technetium with compounds in alkaline solution will produce the oxo-technetium complexes ($TcO(N)_4$), whereas dioxo-technetium complex ($TcO_2(N)_4$) is usually obtained from reaction with compounds in organics solutions such as THF. There are two possible geometric structures for TcO^{3+}, square pyramidal or octahedral. Figure 2 shows that ^{99m}Tc-kanamycin complexes have five coordinate groups with an oxo group at the apex and four nitrogen atoms at the corners so the possible geometric structure for ^{99m}Tc-kanamycin is square pyramidal.

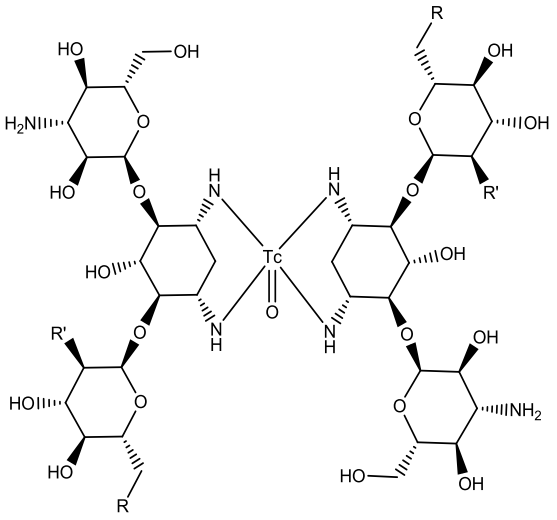
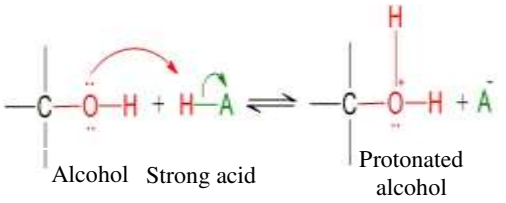


Fig 2. Complex of ^{99m}Tc-kanamycin.

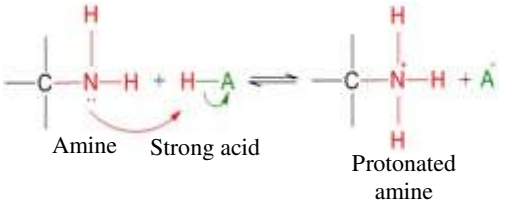
In general, metal salts such as stannous chloride are not soluble in aqueous solution unless the pH is quite low. Stannous chloride acts as reducing agent when dissolved in hydrochloric acid, in which a hexacoordinate tin-chloro coordination complex is formed; the complex keeps the tin soluble (Reaction 1).

$$SnCl_2 + 4\ Cl^- \longrightarrow [\ SnCl_6]^4 \qquad (1)$$

When the pH is raised to between 1.2 and 4.5, the insoluble hydrolysis product tetratin(II) hexahydroxide dichloride, $Sn_4(OH)_6Cl_2$, is formed. At pH higher than 5.5, an amorphous hydrous oxide, $Sn_5O_3(OH)_4$, is formed. The formation of these insoluble hydroxide complexes can be prevented by addition of chelating agents to sequester the stannous ion as a metal chelate [41]. The direct labeling in this study did not use a chelating agent for preventing the formation of the insoluble Sn-hydroxide. Stannous chloride was reacted with kanamycin for a duration before adjusting pH. Moreover, in this experiment stannous chloride was dissolved in hydrochloric acid (5 mg $SnCl_2$ in 0.5 ml 1 N HCl and 4.5 ml 0.1 N HCl) to create an acidic condition. In acidic conditions, the amine and the hydroxyl in kanamycin structure would be converted to protonated form (Reaction 2 and 3). The protonated form of kanamycin would be more positively charged; therefore, it would be easy to bind with $[SnCl_6]^{4-}$. The formation of those chelates will avoid the solution from becoming turbid when the pH is increased.



(2)



(3)

In this preparation, the values of the parameters which influence of the labeling process were varied. Some of those parameters are the amount of reducing agent ($SnCl_2$), the amount of ligand (kanamycin), pH, and incubation time. In this research, a reducing agent for labeling process plays an important role because no effective chemical technique is available to attach a pertechnetate ion to an organic moiety. The reduction of $^{99m}TcO_4^-$ (Tc(VII)) to a lower oxidation state is a prerequisite for ^{99m}Tc-complex formation in high yield and purity [38]. This experiment's result showed that the amount of the reducing agent, $SnCl_2.2H_2O$, which gave the highest labeling efficiency was 30–35 µg. Consequently, a value of 30 µg of $SnCl_2.2H_2O$ was chosen (Fig. 3); it is an optimum value of the reducing agent with the highest labeling efficiency. This suggests that amounts of reducing agents of less than 30 µg were not enough to reduce $^{99m}TcO_4^-$ to a lower oxidation state.

The optimal amount of ligand in the labeling process of ^{99m}Tc-kanamycin is shown in Fig. 4. The highest efficiency was 91.09 ± 0.90 %, attained with 5 mg of kanamycin. This result is the same as the optimum ligand in Roohi's experiment [27]. Radiochemical impurities in this labeling are $^{99m}TcO_4^-$ (free pertechnetate) and $^{99m}TcO_2$ (^{99m}Tc-reduce). The amount of radiochemical impurities present has to be minimized, as a poor radiophamaceutical quality will affect the clinical information. According to the radiochemical purity (RCP) requirement from The United States Pharmacopeia, there are different RCP limit values of the radiopharmaceuticals based on USP monographs, package inserts, and published literature. The minimum RCP limits listed in the USP 27 were >80% [41]. Impurities in the form of $^{99m}TcO_4^-$ will interfere with the imaging of the thyroid region, while $^{99m}TcO_2$ will interfere with the imaging of the liver.

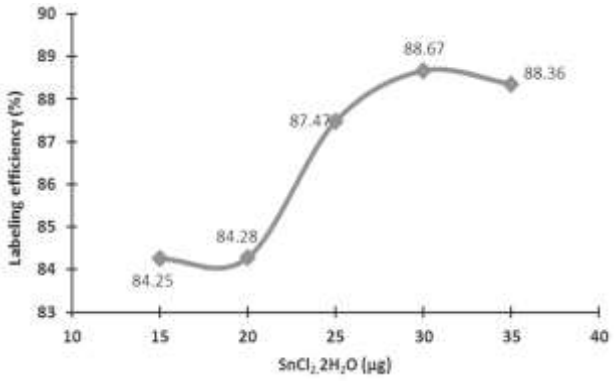


Fig 3. Effect of $SnCl_2.2H_2O$ amount on the labeling efficiency of ^{99m}Tc-Kanamycin.

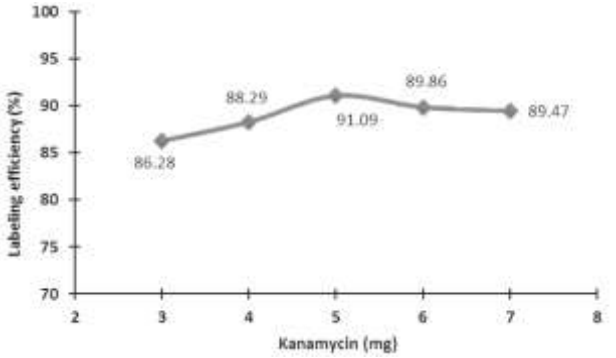


Fig 4. Effect of kanamycin amount on the labeling efficiency of ^{99m}Tc-Kanamycin.

The labeling efficiency of ^{99m}Tc-kanamycin decreases for the use of less than 5 mg of kanamycin (Fig. 4). This result indicates that amounts of the ligand of less than 5 mg was not enough to react with reduced ^{99m}Tc to produce ^{99m}Tc-kanamycin complex. Additionally, from t-test with a confidence level of 95%, for amounts of the ligand of more than 5 mg, the percentages of labeling efficiency were not significantly different with that of 5 mg of the ligand.

As described above, the reaction between $SnCl_2$ and kanamycin took place under acidic condition with pH of about 3. Incubation times between $SnCl_2$ and kanamycin are presented in Fig. 5. It appears that labeling efficiencies of more than 90% occurred for 10-30 minutes of reaction time and 15 minutes of incubation time, respectively. The incubation time was chosen to be 15 minutes since the labeling efficiency for an incubation time of about 15 minutes exceeded 90% (safe zone). Choosing an incubation time of 30 minutes results in the highest labeling efficiency, but it requires care because the labeling efficiency will decrease for incubation times of more than 30 minutes. This decrease is due to the reduction reaction kanamycin by $SnCl_2$. This reaction would decrease $SnCl_2$ reactivity for reducing pertechnetates, as shown by the increasing impurities in the form of $^{99m}TcO_4^-$.

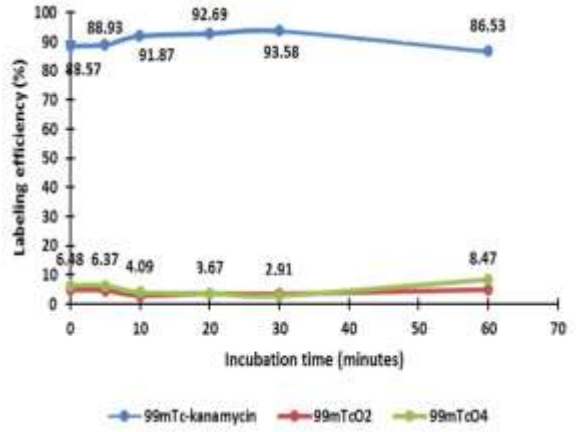


Fig 5. Effect of incubation time before adjust pH.

The effect of pH on the ^{99m}Tc-kanamycin labeling process is shown in Fig. 6. The labelling efficiency increased in alkaline conditions and the highest labeling efficiency was obtained at pH = 9. This optimum condition is suitable for the chemical characteristic of kanamycin, as in alkaline conditions amine or hydroxyl groups would be in deprotonated form. Deprotonated amine and hydroxyl groups have more electrons to be donated than their normal forms, so they will more easily form complexes with ^{99m}Tc to produce ^{99m}Tc-kanamycin. As reported by Roohi *et al*. [27], the complexation of ^{99m}Tc with kanamycin is not rapid and the maximum labeling efficiency is achieved after 30 minutes at room temperature (Fig. 7).

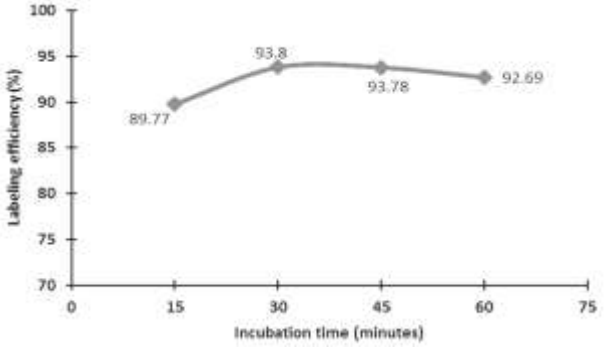


Fig 6. Effect of pH on the labeling efficiency of ^{99m}Tc-Kanamycin.

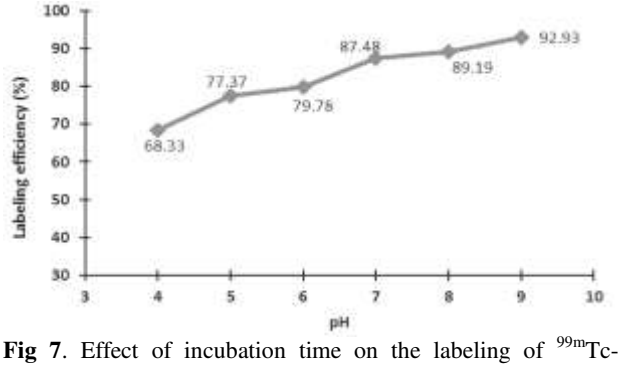


Fig 7. Effect of incubation time on the labeling of ^{99m}Tc-Kanamycin.

CONCLUSION

Kanamycin was successfully labeled with ^{99m}Tc by direct methods, which resulted in a ^{99m}Tc-kanamycin labeling efficiency of 92.31 ± 1.74%. The optimum conditions obtained from this investigation consist of 30 µg $SnCl_2.2H_2O$ as reducing agent, 5 mg kanamycin as a ligand, a pH of 9, and incubation at room temperature for 30 minutes. The preparation is preceded first by reacting stannous chloride with kanamycin for several minutes before adjusting the pH for the labeling process to prevent the formation of insoluble hydroxide complexes of stannous chloride in the alkaline solution.

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REFERENCES

1. E.Y. Sukandar, R. Andrajati, J.I. Sigit *et al.*, ISO Pharmacotheraphy, 2nd ed, PT ISFI Publishing, Jakarta (2009) 965. (in Indonesia)
2. Anonymous, WHO http://www.who.int/mediacentre/factsheets/fs104/en/(2nd September 2015).
3. S. Keshavjee and P. Farmer. The New England Journal of Medecine **367** (2012) 931.
4. M.P.R. Berry, S. Blankley, C.M. Graham *et al.*, Current Opinion In Immunology **25** (2013) 579.
5. N. Fogel, Tuberculosis **95** (2015) 527.
6. A. Doroudi, M. Erfani, M. Rahmatian *et al.*, IOSR Journal Of Pharmacy **4 (12)** (2014) 10.
7. J.R. Tseng, C.W. Lin, S.H. Chen *et al.*, The Journal of Nuclear Medecine **56** (2015) 681.
8. L. Husmann, B.R. Sah, A. Scherrer *et al.*, J. Nucl. Med. **56** (2015) 1024.
9. C. Nanni, C. Errani, L. Boriani *et al.*, J. Nucl. Med. **51** (2010) 1932.
10. A. Signore, A.W.J.M. Glaudemans, F. Galli *et al.*, Bio. Med. Research International **2015** (2015) 1.

11. V. Kumar and D.K. Boddeti, Theranostics, Gallium-68 and Other Radionuclides **194** (2013) 189.
12. F. Rosch, Journal of Postgraduate Medecine **47** (2013) 18.
13. D. İlem-Özdemir, M. Asikoglu, H. Ozkilic *et al.*, Journal of Labelled Compounds and Radiopharmaceuticals **57** (2014) 36.
14. D. İlem-Özdemir, M. Asikoglu and H. Ozkilic, Journal of Radioanalytical and Nuclear Chemistry **298 (**2013) 1635.
15. A. Fazli, M. Salouti, G. Ahmadi *et al.*, Iranian Journal of Medical Physics **9** (2012) 103.
16. T. Ebenhan, J.R. Zeevaart, J.D. Venter *et al.*, J. Nucl. Med. **55** (2014) 308.
17. M.S. Akhtar, M.B. Imran, M.A. Nadeem *et al.*, International Journal of Peptides **2012** (2012) 1.
18. M.H. Sanad and E.H. Borai, Journal of Analytical Science and Technology **5** (2014) 1.
19. S.F. Mirshojaei, M. Erfani, S.E.S. Ebrahimi *et al.*, Iran J. Nucl. Med. **18** (2010) 45.
20. A. Doroudi, M. Erfani, F. Kooshki *et al.*, Iran J. Nucl. Med. **23 (2)** (2015) 96.
21. S.K. Shahzadi, M.A. Qadir, S. Shahzad *et al.*, Arabian Journal of Chemistry **2015** (2015) 1.
22. N. Zainuddin, M.E. Sriyani and E.M. Widyasari, Indonesian Pharmaceutical Magezine **21** (2010) 139. (in Indonesian)
23. I. Dewi, L. Brisman and A.H.S. Kartamihardja, Diagnostic Performance of ^{99m}Tc-ethambutol Scintigraphy In Detecting Peritoneal Tuberculosis (Preliminary Study), Singapore General Hospital Nuclear Medecine Update (2010) 1.
24. K.E. Britton, D.W. Wareham, S.S. Das *et al.*, J. Clin. Pathol. **55** (2002) 817.
25. N. Singh and A. Bhatnagar, Tuberculosis Research and Treatment **2010** (2010) 1.
26. S. Magnet and J.S. Blanchard, Chem. Rev. **105** (2005) 477.
27. S. Roohi, A. Mushtaq, M. Jehangir *et al.*, Journal of Radioanalytical and Nuclear Chemistry **267** (2006) 561.
28. R. Benveniste and J. Davies, Antimicrobial Agent and Chemotherapy **4** (1973) 402.
29. S. Salian, T. Matt, R. Akbergenov *et al.*, Antimicrobial Agent and Chemotherapy **56** (2012) 6104.
30. M. Fosso, M.N. Alfindee, Q. Zhang *et al.*, The Journal of Organic Chemistry **80** (2015) 4398.
31. M.Y. Fosso, Y. Li and S.G. Tsodikova, Med. Chem. Commun. **5** (2014) 1075.
32. C.W.T. Chang and J.Y. Takemoto, Med. Chem. Commun. **5** (2014) 1048.
33. I.M. Herzoge, K.D. Green, Y.B. Zrihen *et al.*, Angew. Chem. **51** (2012) 5652.
34. E.M. Widyasari, N. Zainuddin and W. Nuraeni, Journal for The applications of Isotopes and Radiation **9** (2013) 91. (in Indonesian)
35. E.M. Widyasari, Misyetti, T.H.A. Wibawa, *et. al*, Indonesian Journal of Nuclear Science and Technology **43** (2013) 117. (in Indonesian)
36. E.M. Widyasari, M.E. Sriyani, I. Halimah *et. al,* Ganendra Journal of Nuclear Science and Technology **18** (2015) 1. (in Indonesian)
37. S. Soenarjo, Journal of Radioisotope and Radiopharmaceuticals **17** (2014) 15. (in Indonesian)
38. I. Zolle, Technetium-99m Pharmaceuticals : Preparation and Quality Control in Nuclear Medicine, Springer, New York (2006) 7.
39. G.B. Saha, Fundamentals of Nuclear Pharmacy, 6th ed., Springer, New York (2010) 88.
40. K. Schwochau, Technetium Chemistry and Radiopharmaceutical Applications, Willey-VCH, Weinheim (2000) 145.
41. R.J. Kowalsky and S.W. Falen, Radiopharmaceuticals in Nuclear Medecine, American Pharmacist Association, Washington, D.C. (2004) 1.