The optimization method for synthesis of technetium-99m-luteolin as radiotracer in the development of cancer drugs from flavonoid

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Abstract

The aim of this study is to find the optimum conditions of labeling luteolin flavonoid compounds with technetium-99m (99mTc) to meet the purity requirements stated in the United States Pharmacopeia. This compound is expected to be a potential radiotracer compound for diagnosing cancer. The optimization method in labeling luteolin with technetium determines the parameters such as pH, SnCl₂·2H₂O, genistein concentration, and incubation time. Optimization results of Technetium-99m-luteolin labeling obtained optimum pH conditions 8, the amount of SnCl₂·2H₂O as a reducing agent 60 μL, the optimum amount of luteolin 6 mg/ml, and the optimum incubation time is 30 min. This optimum condition obtained a 99mTc-Luteolin radiochemical purity yield of 94.15%. The radiochemical purity percentage of the 99mTc-Luteolin compound has fulfilled the requirements listed at United States Pharmacopeia, which is ≥90%.

Key words: Luteolin, radiochemical purity, radiotracer, technetium-99m, technetium-99m-genistein

INTRODUCTION

Cancer is a term that describes a disease with a condition where there is uncontrolled cell growth that goes beyond its habits and can spread and attack to other organs. In 2018, cancer is estimated to be the second leading cause of death in the world with 9.6 million cases.[1]

Luteolin is a compound conjugate acid of a 2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxo-4H-chromen-7-olate luteolin-7-olate (1-). Luteolin is one of the potential flavonoids that has been proven to be efficacious as an antioxidant, anti-inflammatory, and for cancer treatment.[2,3] Luteolin is reported to be able to inhibit the catalytic activity of topoisomerase 1 which is a cancer-inhibiting mechanism.[4]

Radiopharmaceuticals are radioisotopes conjugated with biological molecules capable of targeting organs or cells in specific tissues. This radioactive drug can be used for diagnosis and increasingly, for the treatment of diseases. The most widely used radioisotope in diagnostic nuclear medicine is technetium-99m (99mTc). This radioisotope can bind to certain molecules, allowing the diagnosis of many diseases, including certain types of cancer. 99mTc is a radioisotope that can emit pure gamma rays with energy of 140.5 keV, which has a short half-life of about 6 h, does

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not emit charged particle radiation, can be obtained in the form of free carrier, and can bind to many compounds.[5]

The formation of compounds labeled between radioisotopes and ligands for cancer diagnosis purposes must have the specificity to recognize a receptor. Luteolin is an isoflavone compound that has been shown to have anticancer activity through the inhibitory mechanism of topoisomerase I catalytic activity. In humans, the levels of topoisomerase I have been shown to be elevated in colorectal tumors compared to normal colon mucosa.[6]

The purpose of this research was to determine the optimization parameters for the synthesis of $^{99m}$Tc-genistein compounds which have the potential as radiotracer compounds for cancer.

MATERIALS AND METHODS

The tools used in this study were a set of paper chromatography, dose calibrator (Victoreen®), micropipette (Eppendorf®), analytic balance (Mettler Toledo® Type AL204), oven (Memmert®), single channel analyzer (SCA) (ORTEC®), and syringe (Terumo®).

The materials used were luteolin (Sigma-Aldrich®), acetone (Merck®), aquabidestilata (IKA Pharma®), DMSO, HCl 0.1 N, Na. $^{99m}$TcO$_4^-$ (PT. Ansto), Physiological NaCl (IKA Pharma®), NaOH 0.1 N, universal pH indicator (Merck®), KLT SGF-254 (Merck®) plate, instant thin layer chromatography-silica gel (ITLC-SG) (Agilent Technologies®), and SnCl$_2$H$_2$O plates (Sigma-Aldrich®).

Optimization of pH

Determination of the optimum pH conditions on the $^{99m}$TcO$_4^-$ labeling method was carried out using five variations of pH 6, 7, 8, 9, and 10. The composition of each formula is shown in Table 1. Luteolin solution was added with 60 mL of SnCl$_2$H$_2$O solution, then the pH of the solution was adjusted by 0.1N NaOH or 0.1N HCl. After the pH was reached, $^{99m}$TcO$_4^-$ solution was added, and the solution was incubated for 30 min. The solution formed was tested for the purity of the complex formed by dropping it on the thin layer chromatography (TLC) plate SGF-254 and ITLC-SG.[7]

Optimization of SnCl$_2$H$_2$O

In determining the optimum conditions for SnCl$_2$H$_2$O, there is an additional treatment in the form of a vial vacuum. It aims to prevent oxidation by O$_2$ before reacting with $^{99m}$TcO$_4^-$. Determination of the amount of SnCl$_2$H$_2$O as a reductor is done using six variations of the amount of SnCl$_2$ namely 40, 50, 60, 70, 80, and 90 µL with the formula as shown in Table 2 and Figure 1. The test is carried out at the optimum pH, which is pH 8.[8]

Optimization of concentration luteolin

The optimization conditions of the luteolin using five variations of concentration: 3, 4, 5, 6, and 7 mg/mL.

The SnCl$_2$H$_2$O reducing agent was added, and $^{99m}$TcO$_4^-$ amount of 500 µL. Two milliliters of physiological NaCl was added to each solution and then incubated for 20 min. Evaluation of the $^{99m}$Tc-Genistein complex formed is evaluated by measuring the radiochemical purity by dripping each solution on the KLT SGF-254 and ITLC-SG plates.[7]

Optimization of incubation time

The procedure for determining the optimum incubation

| Table 1: The optimum pH and radiochemical purity of $^{99m}$Tc luteolin labeled compounds |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| pH    | Luteolin (µL) | NaOH 0.1N (µL) | TcO$_4^-$ (µL) | Physiological NaCl (µL) | $^{99m}$TcO$_4^-$ (%) | $^{99m}$TcO$_4^-$ (%) | Radiochemical purity (%) | Description |
|-------|---------------|----------------|----------------|----------------------------|----------------|----------------|----------------|-------------|
| 6     | 100           | 5              | 500            | 335                        | 2.29            | 1.25            | 96.46         | Cloudy      |
| 7     | 100           | 10             | 5              | 500                        | 2.70            | 0.16            | 97.15         | Cloudy      |
| 8     | 100           | 15             | 500            | 325                        | 5.56            | 2.63            | 91.81         | Clear       |
| 9     | 100           | 20             | 500            | 320                        | 4.19            | 6.41            | 89.40         | Clear       |
| 10    | 100           | 25             | 500            | 315                        | 0.37            | 91.92           | 7.72          | Clear       |

| Table 2: Composition of formulas for optimization reducing agent of SnCl$_2$H$_2$O |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Luteolin (µL) | NaOH 0.1N (µL) | SnCl$_2$ (µL) | TcO$_4^-$ (µL) | $^{99m}$TcO$_4^-$ (%) | $^{99m}$TcO$_4^-$ (%) | Radiochemical purity (%) | Description |
|----------------|---------------|----------------|----------------|----------------------------|----------------|----------------|-------------|
| 100            | 15            | 40             | 500            | 3.61                        | 8.42            | 87.97         | Clear       |
| 100            | 15            | 50             | 500            | 2.03                        | 1.11            | 96.86         | Clear       |
| 100            | 15            | 60             | 500            | 2.51                        | 0.69            | 96.80         | Clear       |
| 100            | 20            | 70             | 500            | 3.65                        | 1.33            | 95.02         | Clear       |
| 100            | 20            | 80             | 500            | 4.09                        | 1.51            | 94.40         | Clear       |
| 100            | 20            | 90             | 500            | 3.66                        | 8.54            | 87.91         | Clear       |
time for luteolin marking with $^{99m}\text{TcO}_4^-$ used five variations of incubation time, namely 0, 15, 30, 45, and 60 min. Vials that already contain 100 μL luteolin solution and SnCl$_2$.H$_2$O solution are then adjusted to the optimum pH, which is pH 7. The determination of the purity of $^{99m}\text{Tc}$-Luteolin was tested with TLC SGF-254 and ITLC-SG plates.[7]

**Calculation of technetium-99m-luteolin purity percentage**

The method for testing the purity of compounds of $^{99m}\text{Tc}$-Luteolin uses TLC and is measured by SCA. The method consists of a stationary phase TLC SGF-254 and ITLC-SG plates. The mobile phase is ethanol: water: ammonia (2: 5: 1) and NaCl physiological solution. This mobile phase solution is called C$_{1}$.[7]

The equation for calculating the purity of $^{99m}\text{Tc}$-Luteolin compounds is as follows:[9]

\[
\% \text{ of } ^{99m}\text{TcO}_2 (\text{reduced}) = \frac{\text{total number of counts}}{99m \text{Tc-SnCl}_2 \cdot 2\text{H}_2\text{O}} \times 100\%
\]

\[
\% \text{ of } ^{99m}\text{TcO}_4^- = \frac{\text{total number of counts}}{99m \text{TcO}_2} \times 100\%
\]

**Calculation of labeled compounds technetium-99m-Luteolin**

\[
\% \text{ of } ^{99m}\text{Tc-Luteolin} = 100\% - \left(\% \text{ of } ^{99m}\text{TcO}_2 + \% \text{ of } ^{99m}\text{TcO}_4^-\right).
\]

**RESULTS AND DISCUSSION**

The $^{99m}\text{Tc}$-Luteolin compound is formed from a coordinated covalent bond between $^{99m}\text{Tc}$ as a metal and luteolin as a marker or ligand compound. The $^{99m}\text{Tc}$-Luteolin complex formed through the following reactions:

$\text{Luteolin} + \text{Sn}^{2+} + ^{99m}\text{Tc} (\text{VII})\text{O}_4^- \rightarrow ^{99m}\text{Tc}^{(IV)}\text{Luteolin} + \text{Sn}^{4+} + ^{99m}\text{TcO}_2 + ^{99m}\text{TcO}_4^-$

In this reaction, the complex compound $^{99m}\text{Tc}$-Luteolin is produced as the main product, and also $^{99m}\text{TcO}_2$ and $^{99m}\text{TcO}_4^-$ compounds which are radiochemical impurities.[9]

Labeling of luteolin using radioactivity makes it possible to monitor luteolin compounds in the body using a gamma camera that can read radiation exposure provided by technetium. Luteolin is known as a good antioxidant and induces apoptosis in tumor cells and has a valuable effect in cancer prevention and therapy. Luteolin can inhibit the formation of superoxide, namely by inhibiting the activity of xanthine oxidase. In addition, luteolin can also provide antioxidant effects by blocking or producing more endogenous antioxidants such as catalase, glutathione-S-transferase, and glutathione reductase. Therefore, this compound is expected to function as a potential radical scavenging radiotracer.[10] The formation of the $^{99m}$Tc-genistein complex involves electron donors. The structure of luteolin is shown in Figure 2.

The radiochemical purity test of a $^{99m}$Tc-genistein can be measured by the thin layer chromatography method. The stationary phase used is TLC SGF-254 with the mobile phase of physiological NaCl solution. The separation of $^{99m}\text{TcO}_4^-$, $^{99m}\text{TcO}_4^-$ impurity will move toward the peak, while $^{99m}$Tc-genistein will remain at the spot point, ITLC-SG stationary phase with C$_{1}$ mobile phase will separate $^{99m}\text{TcO}_2$. The $^{99m}\text{TcO}_2$ impurity will remain at the spot point, and $^{99m}$Tc-genistein will move toward the peak.[9]

**Test results on pH optimization**

pH conditions are very influential in the formation of the $\text{Tc-Luteolin}$ complex. In addition, the pH conditions will determine the optimum conditions of the SnCl$_2$.2H$_2$O reducing agent. The results for pH optimization testing are shown in Table 1 and Figure 3.

The optimum pH was obtained at pH 7, where the highest radiochemical purity was obtained, which was 97.15%. Under an alkaline pH condition of 8, more TcO$_4^-$ impurities will be produced. At high pH conditions, Sn (II) will be

![Figure 1: The optimum of SnCl$_2$.2H$_2$O and radiochemical purity of Technetium-99m Luteolin](image)

![Figure 2: Chemical structure of luteolin](image)
hydrolyzed, and hence that the ability as a reducing agent decreases. In acidic pH conditions, more impurities will be produced $^{99m}\text{TcO}_2$ because the reducing agent SnCl$_2\cdot$2H$_2$O will reduce more strongly in acidic conditions.$^{[7,8,12]}$

**Test for optimum concentration of SnCl$_2\cdot$2H$_2$O**

The amount of SnCl$_2$, reducing agent used must be sufficient for the reaction to run well. The amount of impurities TcO$_4^-$ and TcO$_2^-$ will affect the purity parameters of $^{99m}\text{Tc}$-Luteolin. The amount of SnCl$_2$ that is too little cannot reduce $^{99m}\text{Tc}^+$ well and hence that it will produce a lot of TcO$_4^-$. Meanwhile, if the amount of SnCl$_2$ is excessive, it can produce a lot of $^{99m}\text{Tc}^4^+$ which will increase the amount of TcO$_2^-$ impurity.$^{[13]}$

Optimization conditions of SnCl$_2\cdot$2H$_2$O solutions were 30 μl with a purity of 90.84% ± 2.38%, with impurities of $^{99m}\text{TcO}_2$ (5.06% ± 1.13%), and $^{99m}\text{TcO}_4^-$ (4.10% ± 0.94%). If the amount of the solution of SnCl$_2\cdot$2H$_2$O is more partial, hydrolyzed SnCl$_2$ forms its hydroxide, and binds with $^{99m}\text{Tc}$-reduced to form $^{99m}\text{TcO}_2$ colloid, so that the amount of impurity will be more $^{99m}\text{TcO}_2$.

**Test for the optimum concentration of luteolin**

The number of ligands (Luteolin) that are not optimal will cause an increase in the number of impurities and reduce the percentage purity of compounds marked $^{99m}\text{Tc}$-luteolin. In testing this parameter, five variations of the amount of luteolin are used: 3, 4, 5, 6, and 7 mg/mL and with the formula shown solutions are in Table 3 and Figure 4.

Optimization of the amount of luteolin with the highest percentage of purity is found in the amount of luteolin 6 mg/mL with a percentage of 94.15%. The use of luteolin amounts <6 mg/mL will reduce the percentage purity of compounds marked $^{99m}\text{Tc}$-Luteolin as the amount of TcO$_2^-$ impurities increases.$^{[9,12]}$

**Test for optimum incubation time**

The duration of incubation time can affect the optimum

![Figure 3: The optimum pH and radiochemical purity of technetium-99m-luteolin labeled compounds](image1)

| Luteolin (mg/mL) | pH | SnCl$_2$ (μL) | NaOH 0.1 N (μL) | Polluter $^{99m}\text{TcO}_2$ (%) | $^{99m}\text{TcO}_4^-$ (%) | Radiochemical purity (%) | Description |
|-----------------|----|--------------|----------------|-----------------------------|------------------------|------------------------|-------------|
| 3               | 8  | 60          | 10             | 8.14                       | 1.99                   | 89.88                  | Clear       |
| 4               | 8  | 60          | 10             | 7.12                       | 1.92                   | 90.96                  | Clear       |
| 5               | 8  | 60          | 10             | 5.63                       | 0.80                   | 93.58                  | Clear       |
| 6               | 8  | 60          | 15             | 5.64                       | 0.21                   | 94.15                  | Clear       |
| 7               | 8  | 60          | 15             | 3.27                       | 32.54                  | 64.14                  | Clear       |

![Table 3: Composition of formulas for optimization of concentration of luteolin](image2)

| Incubation time (min) | pH | SnCl$_2$ (μL) | NaOH 0.1 N (μL) | Polluter $^{99m}\text{TcO}_2$ (%) | $^{99m}\text{TcO}_4^-$ (%) | Radiochemical purity (%) | Description |
|-----------------------|----|--------------|----------------|-----------------------------|------------------------|------------------------|-------------|
| 0                     | 8  | 60          | 10             | 3.31                       | 0.88                   | 95.81                  | Clear       |
| 15                    | 8  | 60          | 10             | 6.60                       | 2.73                   | 91.84                  | Clear       |
| 30                    | 8  | 60          | 10             | 5.09                       | 1.07                   | 93.84                  | Clear       |
| 45                    | 8  | 60          | 15             | 5.23                       | 1.38                   | 93.40                  | Clear       |
| 60                    | 8  | 60          | 15             | 4.20                       | 1.40                   | 94.41                  | Clear       |

![Table 4: Composition of formulas for determining optimization of incubation time](image3)

![Figure 4: The optimum of luteolin and radiochemical purity of technetium-99m luteolin labeled compounds](image4)
formation of compounds marked $^{99m}\text{Tc}$-luteolin. Adequate contact time can affect the reaction between SnCl$_2$, $^{99m}\text{Tc}$ and luteolin. Variations of incubation time used in this study were 0, 15, 30, 45, and 60 min. The formula is shown in Table 4 and Figure 5.

Optimization of incubation time was obtained at 30 min, with a purity percentage of 93.84%. This is because at the incubation time of 30 min is obtained at least from the amount of TcO$_4^-$ impurities.

CONCLUSIONS

Optimization of the conditions in the labeling of the $^{99m}\text{Tc}$-Luteolin compound obtained a 94.15% radiochemical purity, where this condition fulfills USP requirements, which must be ≥90%.

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