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OPTIMIZATION OF PH IN THE LABELING OF APIGENIN WITH TECHNETIUM-99M RADIONUCLIDE AS A POTENTIAL RADICAL SCAVENGING AGENT

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ABSTRACT

Objective. The aim of this research is to determine the pH optimization in the Technetium-99m labeling process with apigenin as a natural flavonoid compound. The antioxidant activity of apigenin can be used to detect the presence of excess free radicals in the body. The compound formed is expected to be a radiotracer compound for cancer diagnosis. **Methods.** The parameter used for optimization is the pH value. Determination of the optimum pH value can be evaluated from the radiochemical purity value of the ^{99m}Tc-Apigenin compound formed. **Results.** The results of pH optimization on the synthesis of ^{99m}Tc-Apigenin compound obtained the optimum pH value is 6 with a radiochemical purity value of 61.35%±4.56%. **Conclusion.** The best pH value for ^{99m}Tc-Apigenin synthesis was 6, with the least amount of impurities $^{99m}TcO_4$ and TcO_2 of 33.77% ± 7.73% and 4.88% ± 3.21%.

KEYWORDS: Apigenin, pH, technetium-99m, radiochemical purity, ^{99m}Tc-Apigenin, radiotracer.

INTRODUCTION

Fast food with high heating and increased pollutants resulting from industrial combustion such as carbon monoxide, nitrogen oxides and hydrocarbons can trigger the formation of free radical compounds that can cause various degenerative diseases such as cancer, stroke, diabetes, myocardial infarction, tissue inflammation, until premature aging.[1]

Free radicals are molecules that are relatively unstable because they have unpaired electrons in the outer shell of their orbitals so they are reactive in finding electron pairs. Free radicals that enter the body will form a chain reaction that produces new free radicals, called oxidative stress. Compounds that can prevent the accumulation of free radicals in the body, or neutralize free radicals are called antioxidants.[2]

Apigenin is one of the natural compounds of the flavonoid class that can be found in some plants and has the function as an anti-inflammatory, antioxidant, and anticancer.[3] Plants that contain apigenin include *Matricaria chamomilla*, onions, parsley, wheat germ, celery (*Apium graveolens*).[4,5] Research that has been done on apigenin compounds proves the existence of antioxidant activity through the mechanism of xanthine oxidase inhibition, as well as disrupting the activity of superoxidase.[6]

The use of radioisotopes that emit gamma rays in apigenin compounds, allows these apigenin compounds to detect the presence of excess free radicals in the body. Technetium-99m is a radioisotope that has a short half-life of around 6 hours, and is often used for diagnostic purposes because it emits pure gamma rays (105.5 keV).[7]

This study aims to determine the optimum pH conditions in the process of labeling Apigenin compounds with Technetium-99m radionuclides.

MATERIALS AND METHODS

Paper chromatography, dose calibrator (Victoreen®), micropipette 5 µL, 10–100 µL, and 100–1000 µL (Eppendorf®), analytic balance (Mettler Toledo® Type AL 204), oven (Memmert®), Single Channel Analyzer(SCA)(ORTEC®), syringe (Terumo®).

The materials used are apigenin(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 $SnCl_2.2H_2O$ (Sigma Aldrich®).

Optimization of pH

Determination of optimum pH conditions in the labeling process of Apigenin with Technetium-99m radionuclides used 5 variations of pH namely pH 4, 5, 6, 7, and 8. Five vials with a size of 10 mL were marked (A, B, C, D, and E) 600 µL of apigenin solution and 30 µL of $SnCl_2.2H_2O$ solution were added. Then, 0.1 M HCl solution or 0.1 M NaOH were added to adjust the pH until the desired pH was obtained, namely pH 4, 5, 6, 7, and 8. After that, each vial was added with a solution of $^{99m}TcO_4^-$ 300 mL and incubated for 30 minutes, and then drop on the Silica gel TLC plate GF-254 and ITLC-SG. The results of determining the purity of complex compounds of ^{99m}Tc-Apigenin at each pH can be calculated and determined.[8]

The Purity Percentage of ^{99m}Tc-Apigenin Compounds

The purity of the compound marked ^{99m}Tc-Apigenin was determined using the thin layer chromatography (TLC) method which was then analyzed using Single Channel Analyzer (SCA). The stationary phase used is the KLT SGF-254 and ITLC-SG plates. For the mobile phase, 2 solvents are used, namely C_1 solution consisting of ethanol: water: ammonia (2: 5: 1) and NaCl physiological solution.[7]

The purity percentage of a compound labeled ^{99m}Tc-Apigenin is calculated based on the percentage of $^{99m}TcO_4^-$ and $^{99m}TcO_2$ (impurity) using the following equation.

$$\%\ 99mTcO2\ (reduced) = \frac{99mTc - SnCl2.2H2O}{\text{total number of counts}} \times 100\%$$

$$\%\ 99mTcO4^- = \frac{99mTcO4}{\text{total number of counts}} \times 100\%$$

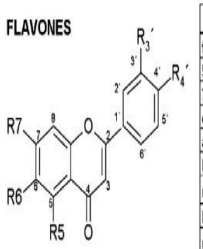
Calculation of labeled compounds ^{99m}Tc-Apigenin.

% ^{99m}Tc-Apigenin = 100% - (% $^{99m}TcO_2$ + % $^{99m}TcO_4$).[9]

RESULTS AND DISCUSSION

Apigenin has been known to have anti-inflammatory effects antioxidants and also inhibits the growth of several types of cancer cells.[10,11] Flavonoid compounds have antioxidant activity because they can stabilize free radical compounds by donating one or more electrons so that free radical compounds do not become reactive.[6]

Complexation of apigenin with technetium-99m is also called radioactive ligand labeling. Labeling apigenin with Technetium-99m allows the presence of apigenin in the body can be monitored by using a gamma camera. The ^{99m}Tc-Apigenin marked compound is formed from the co-ordinated covalent bond between technetium-99m as a metal core and apigenin as a ligand. apigenin will form complexes with Mg metal in the hydroxyl group on C_5 in ring A and the 4-C = O group in ring C.[12]



flavone	R_5	R_6	R_7	R_3'	R_4'
flavone	H	H	H	H	H
5-hydroxyflavone	OH	H	H	H	H
7-hydroxyflavone	H	H	OH	H	H
chrysin	OH	H	OH	H	H
apigenin	OH	H	OH	H	OH
luteolin	OH	H	OH	OH	OH
negletein	OH	OH	OCH_3	H	H
baicalein	OH	OH	OH	H	H
mosloflavone	OH	OCH_3	OCH_3	H	H
diosmin	OH	H	O-Glc-Rha	OH	OCH_3

Fig. 1: Structure of flavones and flavonoid compounds.

The reactions that occur in the ^{99m}Tc-Apigenin complexation reaction are as follows:

Apigenin + Sn^{2+} + $^{99m}Tc^{(VII)}O_4^-$ → $^{99m}Tc^{(IV)}$Apigenin + Sn^{4+} + $^{99m}TcO_2$ + $^{99m}TcO4^-$

From this reaction it is known that in addition to producing ^{99m}Tc-Apigenin labeled compounds, there are also side compounds (radiochemical impurities) in the form of $^{99m}TcO_2$ and $^{99m}TcO_4^-$excess which can affect the purity of compounds labeled ^{99m}Tc-Apigenin.

Optimization results for pH conditions.

The determination of pH optimization is the important factor for the formation of a stable complex in the ^{99m}Tc-Apigenin compound labeling process. Determination of the pH conditions of the solution is done using 5 variations of pH 4, 5, 6, 7, and 8 with the formula shown in table 1.

Table 1: Variation of formulas in pH optimization.

Formula	Apigenin (µL)	pH	$SnCl_2$ (µL)	HCl 0,1 M (µL)	NaOH 0,1 M (µL)	H_2O (µL)	TcO_4^- (µL)	Incubation Time (min)
A	600	4	30	20	-	50	300	30
B	600	5	30	-	-	70	300	30
C	600	6	30	-	40	30	300	30
D	600	7	30	-	50	20	300	30
E	600	8	30	-	60	10	300	30

The results of the five formulas in the pH variations can be seen the average radiochemical purity results shown in Table 2 and Figure 2.

Table 2: Percentage of radiochemical purity of variations in pH.

pH	4	5	6	7	8
Radiochemical Purity	50.14±5.40	54.83±0.36	61.35±4.56	62.95±3.16	51.22±4.82
TcO_2^- (%)	43.01±3.26	36.38±3.79	33.77±7.73	31.24±5.30	21.58±1.4
$TcO4^-$ (%)	6.85±2.28	8.79±3.96	4.88±3.21	5.82±2.22	27.21±5.95

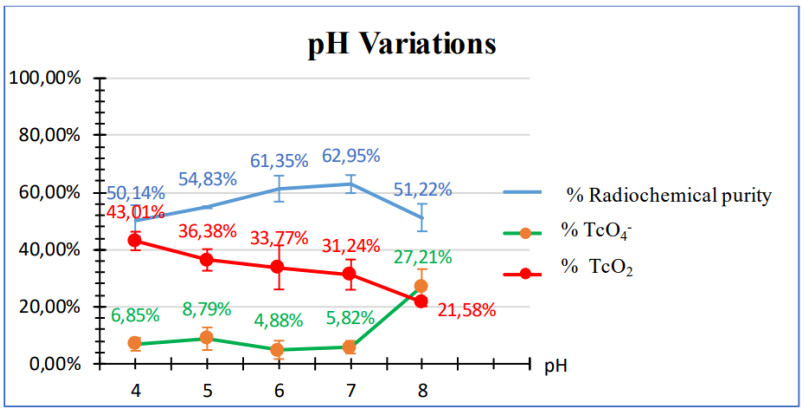


Fig. 2: Radiochemical purity in pH variations.

The result of $^{99m}TcO_2$ impurity percentage in the pH range 4-5 is quite high. This is due to the stronger acidity of the solution, the reactivity of the Sn^{2+} reducing agent to reduce $^{99m}TcO^{4-}$ the higher and the $^{99m}TcO_2$ formed the greater. The excess amount of $^{99m}TcO_2$ will affect the reduction in purity percentage of the ^{99m}Tc-Apigenin complex compound.[8] In addition to acidic conditions, the reactivity of apigenin in forming complexes with metals will decrease.[7]

At pH 8 the percentage of $^{99m}TcO_4^-$ is still quite high. This is due to the weak base condition, the reactor reactivity of Sn^{2+} decreases. The $SnCl_2$ reductant can form colloids, one of them $[Sn(OH)_3]^-$ which can have an influence on the ^{99m}Tc-Apigenin marking.[7] This can be proven by the large percentage of $^{99m}TcO_4$ impurities at pH 8, which is 21.58% ± 5.95%.

The results of optimization of the pH conditions can be at pH 6 with a purity of 61.35% ± 4.56%. This pH 6 is used as the optimum pH in the ^{99m}Tc-Apigenin marking method. Although the percentage of purity at pH 7 is slightly greater than that of pH 6, the percentage of impurity $^{99m}TcO_4^-$ at pH 6 is less. In addition, the reactivity of the $SnCl_2$ reducing agent also decreases with the increase in the pH of the solution.

CONCLUSIONS

The results of the marking of flavonoid compounds with Teknesium-99m radioactive compounds obtained the optimum pH conditions at pH 6 with radiochemical purity of 61.35% ± 4.56%.

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