Hydroxyapatite (Ha) labeling with a phosphorus-32 radioisotope of the TRIGA 2000 reactor irradiation result as a candidate for radiosinovectomy therapy

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Abstract. Radiosynovectomy is a therapy performed on patients with acute-level arthritis (rheumatoid arthritis) as an alternative solution besides surgery. Radiosynovectomy is performed using a labeled compound with a particle size of 0.5 - 10 µm labeled with a β radioisotope. Hydroxyapatite (HA) is a 1-10 µm-sized compound found in bones with the components of Calcium (Ca) & Phosphorus (P). Phosphorus-32 (³²P) is a radioactive form of Phosphorus which emits pure beta rays and is often used for therapy. Labelling HA with ³²P tends to be easy to do with a substitution reaction, because phosphorus is the main constituent of HA. Phosphorus-32 was made by irradiating natural sulfur at the Bandung TRIGA 2000 reactor facility following the ³⁵S (n, p) ³²P reaction mechanism. The separation process of Phosphorus-32 was carried out by a distillation method followed by extraction with 0.01 N HCl accompanied by heating for 30 minutes. The Phosphorus-32 solution is then passed through a 3 gr cation exchange resin. Before Phosphorus-32 solution was used for Labelling of HA, a Radionuclide Purity test was performed with a gamma-MCA spectrophotometer and a Radiochemical Purity test using paper chromatography. The test results showed Phosphorus-32 had Radionuclide Purity > 99.99% and Radiochemical Purity > 96%. 0.5 mCi Phosphorus-32 which meets the quality test requirements is reacted with 7 mg Ha at pH 7. Then it is vortexed at 1500 rpm for 60 minutes with 70 °C heating. HA-³²P is separated using centrifugation into residual and supernatant fractions. Measure the radioactivity of both fractions with a dose calibrator. Labeling Yield HA with Phosphorus-32 was obtained 98%. Furthermore this HA is ready to be used in in vivo tests for radiosynovectomy.

1. Introduction

Phosphorus is one of the most important elements in the world with abundance on earth of 0.099% and abundance in the human body of 1.1%. With a large enough abundance, phosphorus has an important role in life, starting from as a basic ingredient in the formation of DNA-RNA in the human body to phosphorus in the form of radioisotopes. The most widely used radioisotope-shaped phosphorus is phosphorus with mass number 32 or commonly called ³²P. In agriculture, Radioisotopes Phosphorus is commonly used to evaluate the distribution of minerals in plants. First, the radioisotope phosphate is sown to the ground, then it can be checked for fertilizer distribution in plants by analyzing the activity of the phosphorus which is distributed to the parts of the plant [1]. in the field of industry, Phosphorus-32 can be used in the cosmetic industry as an ingredient in removing scars that arise (Keloid) [2].
Whereas in the health sector Phosphorus-32 which is a pure beta transmitter is commonly used as a therapy (radiotherapy) for cancer patients [3].

The Bandung TRIGA 2000 reactor since the 80s has produced Phosphorus-32 irradiated from Sulphur following the reaction mechanism $^{32}\text{S} (n, p) ^{32}\text{P}$, then it is separated from the rest of Sulphur using the extraction method. Phosphorus-32 itself is a radioisotope that transmits pure beta energy with 695 keV energy and a half-life of 14.3 days [4]. However, the extraction method that has been used in PSTNT has a lack of radiochemical purity, which is represented by the percentage of orthophosphate obtained which is not good enough and the polyphosphate and pyrophosphate impurities contained are still quite high. The yield in the form of activities obtained from the process is also less than optimal [4] [5]. Therefore it is necessary to do a separation process with a better method to improve the quality of this Phosphorus-32 product. The distillation-extraction method is a newer method and is a refinement of the previous method. By using this distillation-extraction method it is hoped that it can increase the activity of the product, minimize unwanted phosphorus impurities and make the $^{32}\text{P}$ product meet the requirements to be made into a Labelled Compound.

Hydroxyapatite (HA) belongs to the group of Calcium phosphate salt (CaP) which is the main mineral that forms bone (50 - 75% of bone weight) and teeth. HA has the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ which can be made by reacting compounds made from Ca ($\text{CaCl}_2$, $\text{Ca(OH)}_2$, $\text{Ca(NO}_3)_2$) with Phosphate ($\text{KH}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$) compounds at temperatures height [7] [8]. HA has a size between 1-10 µm and has a monoclinic crystal structure (the result of a stoichiometric reaction with a Ca / P composition of 1.67) or hexagonal (the result of a non stoichiometry reaction) [7]. HA can also be used as a material for marking intracellular proteins [8], a target for fluorescence imaging [9]. HA is also often reacted with radioisotopes into labeled compounds used for a therapy called radiosynovectomy [10] [11].

Radiosynovectomy in general is a therapy aimed at patients with acute arthritis (rheumatoid arthritis) as a solution other than surgery. This therapy uses a β radioisotope transmitter that is injected through the gaps of the joint (Intra Articular) to the joint fluid (synovial). Another requirement of the labeled compound to be injected is to have a particle size ranging from 0.5 - 10 µm [12]. from the compound criteria for radiosynovectomy over compounds labeled Hydroxyapatite labeled Phosphor-32 (HA,$^{32}\text{P}$) is very promising to use, both because of its easy synthesis, particle size that meets and is easily reacted with β-phosphorus radioisotope transmitters.
2. Materials and Methods

2.1. Materials

The materials used in this study include: Sulphur (Merck), 37% Hydrochloric Acid, Aquadest, 50 Wx8 Dowex Cation Exchanger Resin, Whatman I Paper, PEI (Polyethylenimine) Cellulose Plate, Hydroxyapatite, Isopropyl alcohol, Trichloracetic Acid 50%, NH$_4$OH 25% and KH$_2$PO$_4$ [4] [6].

2.2. Apparatus

While the equipment used includes: A set of glass tools for extraction distillation, dose calibrator, gamma-MCA spectrophotometer, Geiger Muller detector (GM), vortex mixer and a set of paper chromatography tools [4] [6].

2.3. Production of Radioisotope Phosphorus-32

At first Sulphur irradiated by the TRIGA Bandung reactor with a flux of $\sim 10^{13} \text{n/cm}^2\cdot\text{s}$ was placed in a three-neck flask and then heated. When the heating process is carried out 2 kinds of variations, first with the extraction method and the extraction distillation method. In the Sulphur extraction method, it is heated at 120°C until it melts and the color change to reddish-brown. Then 100 ml of the eluent is added to the flask and heating continues until the specified time. catch the eluent from the pumpkin into the vial and repeat the step again. The results obtained are 2 vials eluted. While in the distillation process, Sulphur in the flask is heated to a temperature of 300°C. in this condition the Sulphur will evaporate while the phosphorus will be left in the pumpkin. Capture Sulphur vapor into a flask filled with water with a vacuum system. Sulfur has been Distilled for 3 hours until it was used up. After the distillation end, remove the distillation device from the three-neck flask. done the extraction process by adding 100 ml eluent and heat it for a certain time. the phosphorus solution transferred into the vial and repeats the elution process once again.

Time variation for extracting was 30 minutes, 45 minutes and 60 minutes. The HCl solutions ware used for extractant with variation of normality were 0.01; 0.1; 0.5; 1. After obtaining a Phosphorus-32 solution, the solution is passed into the resin column, the resin is prepared by immersing it with 0.1 N HCl for 1 hour. Then wash with distilled water until the pH of the resin rises to 5. The variation of the resin weight is 1 gr, 3 gr and 5 gr. After the phosphorus solution is passed in the resin column, collect the end product in vials and give an information sticker.

2.4. Quality Control Test

Radionuclide Activity Testing uses a Dose Calibrator with a NaI (Tl) detector. the vial containing Phosphorus-32 solution Insert to the sample container, and selected Phosphorus-32 in the menu screen. The results of the activities can be seen on the screen.

Radiochemical purity was tested by paper chromatography and thin-layer chromatography. In paper chromatography, the stationary phase used is Whatman I paper with a length of 23 cm and given a number from -2 to 20. As a mobile phase, a mixture of isopropyl alcohol is used: Aquadest: Trichloracetic acid 50%: Ammonia solution 25% with a ratio of 75:15: 9.5: 0.5. Then measure the activity per centimeter of paper with the Geiger Muller tool.

Whereas in the thin layer chromatography method, a PEI (Polyethylenimine) Cellulose plate was used as a stationary phase along 20 cm. the mobile phase used is a 1 M KH$_2$PO$_4$ solution with Ph 3.5.
Radionuclide purity Analysed using a Gamma Spectrophotometer-Multi Channel with HPGE detector. Take 5 µl Phosphorus-32 in a vial. Analyzer for 1 hour.

2.5. Synthesis of Labelled compounds HA$^{32}P$

Prepare Hydroxyapatite with a weight variation of 1-10 mg into the microtube, each added 0.5 mCi radioisotope P-32 solution. Adjust the pH of the solution with a variation of 5, 6 and 7. Add NaCl solution to 1 mL, then vortex the solution at 1500 rpm with variations of time 15, 30, 45 and 60 minutes. Heat with a temperature variation of 50, 60, 70 and 80 °C for 30 minutes. The solutions were Centrifugated for 5 minutes at 5000 rpm, and it separated the residual and supernatant fractions. After all, the end product was Measured activity in both Dose Calibrator and Wipe Counter [11].

3. Results and Discussion

From the results of experiments have been successfully produced radioisotope solution Phosphorus-32 using various variables to determine the optimum state. First the production process is carried out using the extraction method compared to the distillation-extraction method. This experiment used a constant variable in the form of 0.01 N HCl concentration and extraction time of 30 minutes.

| Method                  | Poly phosphate | Pyro phosphate | Orthophosphate |
|-------------------------|----------------|----------------|---------------|
| Extraction #1           | 15.70%         | 6.40%          | 77.90%        |
| Extraction #2           | 20.60%         | 1.20%          | 78.20%        |
| Distillation-extraction #1 | 13.60%       | 0.00%          | 86.40%        |
| Distillation-extraction #2 | 13.10%       | 0.30%          | 86.60%        |

Table 2. Radioactivity & Yield of Extraction & Distillation-extraction method

| Method                         | Initial Radioactivity | Obtained Radioactivity | % Yield |
|-------------------------------|-----------------------|------------------------|--------|
| Extraction (µCi)              | 1241.1                | 249.8                  | 20%    |
| Distillation-extraction (mCi) | 51.14                 | 22.27                  | 44%    |

the experimental results can be seen in table 1, that the orthophosphate yield obtained from the distillation-extraction process was 86.4% and 86.6%, greater than the extraction method which only received 77.9% and 78.2%. For radioactivity of Phosphorus-32 in the extraction method can be seen in table 2, from the initial activity - 1241.1 µCi obtained 249.8 µCi results (20%). While the results of the distillation-extraction method obtained 22.27 mCi results from the radioactivity of 51.14 mCi (44%).
From these experiments it can be seen that the distillation-extraction method produces better orthophosphate yield and activity. [6]

Next is the optimization of the eluent concentration and the optimum heating time at the time of extraction. Firstly the experiment was carried out with a variable HCl concentration which was eluent during the extraction process. Time and temperature are set constant at 30 minutes & 80 ° C on all variables.

| The concentration of HCl (N) | Started Radioactivity (µCi) | Obtained Radioactivity (µCi) | % Yield |
|-----------------------------|----------------------------|------------------------------|---------|
| 0,01                        | 140                        | 109                          | 78%     |
| 0,1                         | 140                        | 83                           | 59%     |
| 0,5                         | 140                        | 101                          | 72%     |
| 1                           | 140                        | 106                          | 76%     |

The results of the experiment were presented in table 3 and show that the best eluent is HCl with a concentration of 0.01 N [4]. To determine the optimum time, the temperature used is set constant at 80 ° C and the optimum concentration obtained is 0.01 N for all time variables.

![Graphic of Optimalitation extraction time](image_url)

**Figure 2.** Yield from extraction time variation

From the graph of optimization extraction time in figure 2, although at a glance variable extraction time of 30 minutes obtained the most optimal results, when viewed as a whole from the purity of
orthophosphate obtained and the yield of the activity found no significant differences. So the conclusion is that the time used is the fastest time, 30 minutes.

After P-32 has been successfully separated from the irradiated Sulphur, the radioisotope solution must be passed through the resin column to remove impurities in the form of pyrophosphates and polyphosphates as well as innate impurities contained in the Natural Sulphur target. Experiments using a variety of resin weight 1, 3 and 5 grams [4].

![Figure 3. Purity from resin weight variation](image)

The results obtained from experiments were presented in figure 3, that by being passed in the orthophosphate purity resin from the Phosphorus-32 solution can be increased and the polyphosphate and pyrophosphate impurities are reduced in percentage. From the graph it can be seen that the best initial increase in orthophosphate purity from 79.2% to 96.3% was obtained with the use of resin weight 3 & 5 grams, however the addition of using 5-gram resin was no significant impact, so the conclusion was drawn, that the most optimum weight of elution use 3 grams of resin.

After the optimum conditions of the Phosphorus-32 production process are obtained by the distillation-extraction method, the HCl concentration as an eluent is 0.01 N, the extraction time is 30 minutes and the weight of the receipt used is 3 grams, a series of quality test procedures are carried out on the Phosphor-32 Solution. First the pH test of the solution is carried out using a universal pH indicator and a result of 2. Then the radionuclide impurity is checked using the Gamma Spectrophotometer - Multi-Channel Analyzer and the results have been seen in figure 4 [4].
Figure 4. Gamma spectrum of P-32 on Gamma Spectofotometer-MCA

Possible impurities can be formed from natural Sulphur irradiation both activated by fast neutrons and thermal neutrons can be seen in table 4 [6].

Table 4. Sulphur Side reaction in the nuclear reactor

| Isotope of Sulphur | abundance (%) | Activation products formed | Fast neutrons | Thermal neutrons |
|--------------------|--------------|----------------------------|---------------|-----------------|
|                    |              | Reaction                   | Half-Life     | Reaction        | Half-Life        |
| $^{32}\text{S}$   | 95.02        | $^{32}\text{S}(n,p)^{32}\text{P}$ | 14.26 d       | $^{32}\text{S}(n,\gamma)^{33}\text{S}$ | Stable          |
| $^{33}\text{S}$   | 0.75         | $^{33}\text{S}(n,p)^{33}\text{P}$ | 25.3 d        | $^{33}\text{S}(n,\gamma)^{34}\text{S}$ | Stable          |
| $^{34}\text{S}$   | 4.21         | $^{34}\text{S}(n,p)^{34}\text{P}$ | 14.4 s        | $^{34}\text{S}(n,\gamma)^{35}\text{S}$ | 87.2 d          |
| $^{36}\text{S}$   | 0.02         | $^{36}\text{S}(n,p)^{36}\text{P}$ | 5.9 s         | $^{36}\text{S}(n,\gamma)^{37}\text{S}$ | 5.05 m          |

Radionuclide purity testing by paper chromatography obtained the following results

Figure 5. Radiochemical test from P-32, (a) Using Paper Chromatography (b) Using TLC

From the result in figure 5, radiochemical yet using paper chromatography and Thin layer chromatography obtained orthophosphate content more than 95%. The radioisotope results were passed
requirements that will be used for the synthesis process with HA. HA is made by using a concentration ratio of 1-10 μg. Then the pH 5-7 is used, the vortex time is 15-60 minutes and the last variable used is the temperature at the time of the reaction at 60-80 ° C. The results of the four variable variables are seen in the graph below:

![Graphical representation of the results](image)

**Figure 6.** Graphics Yield Labelled Hydroxyapatite with P-32 Vs Weight of HA (a); pH of reaction (b).

Radioactivity yield was measured using a GM Counter counter and ROI Counter facility on the Dose Calibrator plus wipe the counter. From the result in figure 6, it was found that the highest yield was obtained at 7 mg HA weight. If only in terms of the results of GM counters there were no significant differences, but if the yield data were combined with the ROI Count method, a significant difference could be seen and concluded that 7 mg was the optimum weight during the reaction.

The optimum pH when the HA reaction with Phosphorus-32 is 7. This occurs because initially the radioisotope Phosphorus-32 has a structure of $\text{H}_3\text{[}^{32}\text{P}]\text{PO}_4$ and pH 2, when neutralized with NaOH until pH 7 the structure changes to $\text{Na}_3\text{[}^{32}\text{P}]\text{PO}_4$ which is the best form when marking HA-32P [11].

![Graphical representation of the results](image)

**Figure 7.** Graphics Yield Labelled Hydroxyapatite with P-32 Vs vortex time (c); Temperature of Reaction (d)

During the HA-32P reaction was stirred using vortex with an optimum time of 60 minutes and heating with a temperature of 70 ° C in accordance with the results of variations that can be seen in graphs (C) & (d) at figure 7. This result also reinforces the previous theory which says that the optimum time for the reaction is 60 minutes and if it is increased again the results obtained were not significant [11]. In the variable temperature seen an upward trend starting from room temperature to a temperature of 70 °
C, and concluded that the increase in yields ranging from room temperature to 70 ° C has a positive and optimum trend at a temperature of 70 ° C.

4. Conclusion

Radioisotope Phosphorus-32 successfully produced with a purity > 98%, no radionuclide impurities obtained and pH of solution 2. The radioisotope has been successfully used as a material to labeling hydroxyapatite with the highest yield obtained at 98%. From this research, an optimal method of producing phosphorus-32 radioisotope and HA-P$^{32}$ labeling can be obtained as a reference for subsequent research and production.

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References

[1] Meyer G, Maurhofer M, Frossard E, Gamper H A, Mäder P, Mészáros, Schönholzer-Mauclaire L, Symanczik S and Oberson A 2019 Pseudomonas protegens CHA0 does not increase phosphorus uptake from 33 P labeled synthetic hydroxyapatite by wheat grown on calcareous soil Soil Biol. Biochem. 131 217–28
[2] Rahman W Y, Sarmini E, Herlina H, Abidin A, Triyanto T, Hambali H, Sriyono S and Widyaningrum T 2019 Preliminary Study on Production Of 32P–Labeled Phosphate Chromic as A Material for Skin Patch Indones. J. Pharm. Sci. Technol. 1 26
[3] Bhutani M S, Cazacu I M, Luzuriaga Chavez A A, Singh B S, Wong F C L, Erwin W D, Tamm E P, Mathew G G, Le D B, Koay E J, Taniguchi C M, Minsky B D, Pant S, Tzeng C W D, Koong A C, Varadhachary G R, Katz M H G, Wolff R A, Fogelman D R and Herman J M 2019 Novel EUS-guided brachytherapy treatment of pancreatic cancer with phosphorus-32 microparticles: first United States experience VideoGIE 4 223–5
[4] International Atomic Energy Agency IAEA-TECDOC-1340 2003 Manual for reactor produced radioisotopes 1–254
[5] Rahman W Y 2012 Pembuatan Radioisotop Fosfor-32 untuk Sintesa ATP Bertanda 32P [(Y-32P)ATP] Pros. Semin. Pengelolaan Perangkat Nukl. Pus. Teknol. Akselerator dan Proses Bahan Yogyakarta ISSN 1410–1137
[6] Vimalnath K V., Shetty P, Rajeswari A, Shirayil V, Chakraborty S and Dash A 2014 Reactor production of 32P for medical applications: An assessment of 32S(n,p)32P and 31P(n,γ) 32P methods J. Radioanal. Nucl. Chem. 301 555–65
[7] Lin K and Chang J 2015 Structure and properties of hydroxyapatite for biomedical applications vol 4214 (Elsevier Ltd.)
[8] Vázquez-Hernández F, Mendoza-Acevedo S, Mendoza-Barrera C O, Mendoza-Álvarez J and Luna-Arias J P 2017 Antibody-coupled hydroxyapatite nanoparticles as efficient tools for labeling intracellular proteins Mater. Sci. Eng. C 71 909–18
[9] Chen F, Huang P, Zhu Y J, Wu J and {Citation} Cui D X 2012 Multifunctional Eu 3+/Gd 3+ dual-doped calcium phosphate vesicle-like nanospheres for sustained drug release and imaging *Biomaterials* **33** 6447–55

[10] Gallant R, McNall-Knapp R Y and Khan O 2019 Remote arterial vasculitis as a possible complication of Phosphorus-32 Radiosynovectomy *Radiol. Case Reports* **14** 137–40

[11] Rajeswari A, Vimalnath K V., Sarma H D, Shetty P, Mohammed S K, Nuwad J, Chakraborty S and Dash A 2016 Hydroxyapatite (HA) microparticles labeled with 32P – A promising option in the radiation synovectomy for inflamed joints *Appl. Radiat. Isot.* **116** 85–91

[12] André V, Dalibard V, Dernis E, Varin S and Cormier G 2018 Current role for radioisotope synovectomy *Jt. Bone Spine* **85** 295–9