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Preliminary Study on Production Of ^{32}P – Labeled Phosphate Chromic as A Material for Skin Patch

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Abstract

Keloids are skin disorders or benign tumours that are due to abnormal wound healing in the binding tissue after trauma, inflammation, surgical wounds, or burns. Low activity radioisotopes have shown to be effective in curing or eliminating keloids on the skin. One of these radioisotopes is phosphorus-32 (^{32}P), a beta (β^-) emitter with a half-life of 14.3 days. This radioisotope can also be developed for the treatment of keloid and skin tumours. Currently, the keloid is treated by conventional methods where a filter paper which has been wetted with ^{32}P in form of its sodium phosphate directly applied on the area of the keloid. However, this method is considered inefficient and less secure. The purpose of this research is to obtain technology for preparing of ^{32}P-labeled skin patch which is expected to be which will not decompose easily. The first step of this research is to produce ^{32}P-labeled chromic phosphate ($Cr^{32}PO_4$) colloids, a precursor of skin patch, through condensation which involves oxidation-reduction reaction. In this step, Cr (VI) is reduced to Cr (III) to form $Cr^{32}PO_4$ with a particle size of <1 μm. These particles ($Cr^{32}PO_4$) are expected to evenly distributed when mixed with silicon to form skin patch. The reaction gave a yield of 97,8%. The results of characterization show that the prepared $Cr^{32}PO_4$ colloids have a particle size of > 1μm. Further study needs to be performed in due time in order to have $Cr^{32}PO_4$ colloids with suitable particle size.

Keywords: Keloid, Chromic phosphate colloid, Skin patch, Condensation, Oxidation-reduction reaction

1. Introduction

Radioisotope ^{32}P has long been used for therapy in nuclear medicine. In radiotherapy, ^{32}P administered through a medium of catheter, wire, and seeds. Phosphorous-32 can be produced in a reactor with a nuclear reaction of $^{32}S(n,p)^{32}P$. Phosphorous-32 is β-particle transmitter that generates maximum energy of 1.71 MeV with an average energy of 0.7 MeV and a half-life of 14.2 days [1]. The range of beta particle maximum distance in air is 620 cm in air, 0.8 cm in tissue and 0.6 cm in plexiglass Phosphorous-32 as a beta transmitter has a low linear energy transfer (LET) compared to alpha particles[2]. Therefore, ^{32}P is considered suitable for the treatment of keloid and skin tumours. Up to now, there is no keloid therapy that gives satisfactory results. Combination therapies include surgery and radiotherapy are considered the best therapy for keloids that do not respond to conventional therapy[3]. Salgueiro (2009) has conducted research to see whether the effects of radioisotopes as open sources can have the same effect as a sealed source[4]. In this study, ^{32}P solution was converted into chromic phosphate powder which was then mixed with silicon to form ^{32}P sheets or plaster. The results of this study indicate that open source ^{32}P meets the bio-evaluation requirements for design as

brachytherapy material in keloids[56]. Although this ^{32}P sheets is in form of an open source its nature work resembles a sealed source. It needs to be noted that radioisotope ^{32}P therapy for keloid / benign skin tumors has never been in the form of a sealed source. Treatment of keloids using radioisotope ^{32}P can minimize the effects of recurrence after therapy[7].

Preparation of radiolabeled chromic phosphate ($Cr^{32}PO_4$) colloid can be done in 2 ways, namely dispersion and condensation. In dispersion method, large particles are destructed into small particles, the size of colloidal particles[8]. While in the condensation method, small ion molecules chemically enlarged to the size of colloidal particles. One of several techniques for molecular enlarging is by an oxidation-reduction reaction. Preparation of radiolabeled chromic phosphate colloids is carried out by condensation method using a redox reaction. In this reaction sodium sulphite used as a reducing agent, where Cr (VI) in form of chromic acid reduced to Cr (III) to form chromic phosphate ($CrPO_4$) with a phosphatic acid containing radioisotope ^{32}P[9]. The formed chromic phosphates-32 ($Cr^{32}PO_4$) then used as active ingredients for skin patches.

Therefore, the aim of this project is to develop a reliable procedure for preparation $Cr^{32}PO_4$ used as active ingredients for making radioisotope ^{32}P radiolabeled skin patches for the treatment of keloids. One of $Cr^{32}PO_4$ precursor is ^{32}P self-produced in form of $H_3{}^{32}PO_4$ by the Centre for Radioisotope and Radiopharmaceutical Technology, Nuclear Energy Agency (PTRR - BATAN), Serpong. It is expected that from this development it can be obtained colloidal compounds of $Cr^{32}PO_4$ which conforms to the required specification. The $Cr^{32}PO_4$ in due time would be a further process to form ^{32}P radiolabeled skin patches which can be then used for n in the treatment of keloids/skin tumors in hospitals in Indonesia.

2. Materials and Methods

The $Cr^{32}PO_4$ colloid was synthesized using condensation method involving an oxidation-reduction (redox) chemical reaction from orthophosphate compounds containing ^{32}P with chromate and gelatine. As the reducing agent, sodium sulfite will reduce Cr (VI) to Cr (III) to form $Cr^{32}PO_4$ colloidal compounds. The formed colloid was added to a gelatine solution, a colloid protector.

2.1. Materials

Materials used in this project include chromic (VI) oxide (SigmaAldrich), orthophosphoric acid from Sigma, (^{32}P) as ($H_3{}^{32}PO_4$), a in-house produced by Radioisotope Production Technology Division, Centre for Radioisotope and Radioisotope Technology (PTRR) – National Nuclear Energy (BATAN), gelatine solution 2% (Sigma), aquabidest (API – IPHA), potassium dihydrogen phosphate (KH_2PO_4) and sodium sulfite (E.Merck), PEI cellulose strips, (E.Merck).

2.2. Equipment

Equipment used in the project included distillation equipment, thermometer (Alfa France), hot plate stirrer (As One Rexim RSH), a chamber for analysis of thin layer chromatography (TLC), autoradiography scanner (Cyclon Plus Perkin Elmer), Particle Size Analyser (PSA, Horiba LB-550).

2.3. Methods

In early stages, preparation of $Cr^{32}PO_4$ colloids performed by refluxing a mixture of 6 mL of chromic acid 0.4 mM (10 mg/mL) and 4 mL of phosphate acid 0.4 mM (10 mg/ml) containing radioactive phosphate ($H_3{}^{32}PO_4$) in a water bath at 80°C for 5 minutes. Aquabidest (6.5 mL) then added which followed by addition of 0.5 mL gelatine solution (2 %) and 1 mL of sodium sulphite 0.16 mM (200 mg/mL). In its final stage, the mixture was stirred at 500 rpm for 20 min at 80°C.

2.4. Analysis

Particle size of colloidal $Cr^{32}PO_4$ was analyzed using PSA. Formation yield of colloidal $Cr^{32}PO_4$ determined using thin layer chromatography (TLC) where PEI cellulose used as a stationary phase and KH_2PO_4 solution used as a mobile phase and visualization using autoradiography scanner Cyclon Plus Perkin Elmer.

3. Result

Prior to the synthesis of $Cr^{32}PO_4$ colloids, $H_3{}^{32}PO_4$ solution was tested to determine its radiochemical purity. Figure 1. shows the radiochromatogram of $H_3{}^{32}PO_4$ solution used for the synthesis of $Cr^{32}PO_4$ colloids. It can be seen from Figure 1. $H_3{}^{32}PO_4$ moved with the mobile phase to give an Rf of 0.687 and the percentage of radioactivity under this peak reached 98.2%. This result indicated that $H_3{}^{32}PO_4$ solution has a radiochemical purity of 98.2%.

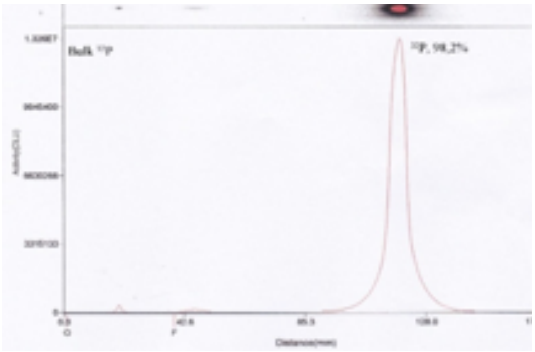
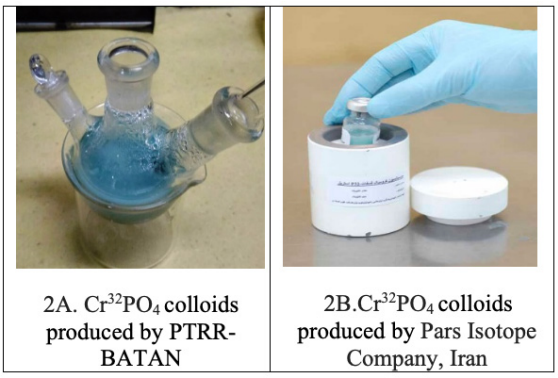


Figure 1. Radiochromatogram $H_3{}^{32}PO_4$ solution

Figure 2 shows $Cr^{32}PO_4$ colloids that have been prepared using condensation method which involves oxidation-reduction reaction. $Cr^{32}PO_4$ colloid was prepared using 5,991 mCi of $H_3{}^{32}PO_4$ solution. It can be seen that $Cr^{32}PO_4$ colloids prepared made by PTRR-BATAN resembled to $Cr^{32}PO_4$ colloids prepared by Isotope Company Pars, Iran. This result suggested that the synthesis process $Cr^{32}PO_4$ colloids carried out at PTRR-BATAN might have been already formed.

The yield of synthesized $Cr^{32}PO_4$ colloids analyzed using TLC where PEI cellulose used as the stationary phase and



2A. $Cr^{32}PO_4$ colloids produced by PTRR-BATAN

2B.$Cr^{32}PO_4$ colloids produced by Pars Isotope Company, Iran

Figure 2. $Cr^{32}PO_4$ colloids

KH_2PO_4 solution used as the mobile phase. Radiochromatogram of this analysis is shown in Figure 3. Unlike $H_3{}^{32}PO_4$ solution, a starting material, $Cr^{32}PO_4$ colloids stayed at the origin (the area where it was spotted) to give a R_f of 0.125 (Figure 3). Percentage radioactivity under this peak which indicate the percentage of the yield as well radiochemical purity of $Cr^{32}PO_4$ colloids reached 97.8%.

The particle size analysis of $CrPO_4$ colloids was performed using PSA. The PSA analysis result of $Cr^{32}PO_4$ colloids shown in Figure 4.

It can be seen from Figure 4. that the particle size of the formed $CrPO_4$ colloids ranges between 3 - 5 µm in diameter (3000 - 5000 nm) reached 58.98%. The desired particles size $Cr^{32}PO_4$ colloids, however, are 0.1 - 0.6 µm in diameter (100 - 600 nm).

Synthetic optimization for formation of $Cr^{32}PO_4$ colloids which have a particle size of 0.1 - 0.6 µm was then performed. Optimization was performed by varying the stirring speed during the reflux process. The PSA analysis results of these $Cr^{32}PO_4$ colloids, showed in Figure 5.

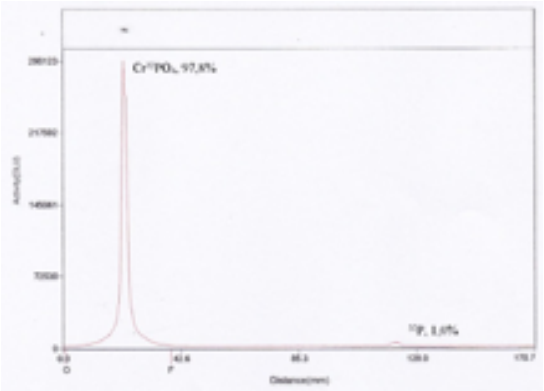


Figure 3. Radiochromatogram of $Cr^{32}PO_4$

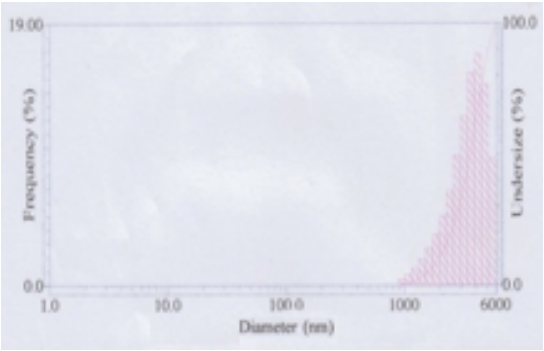


Figure 4. Results of analysis of $CrPO_4$ colloids with PSA

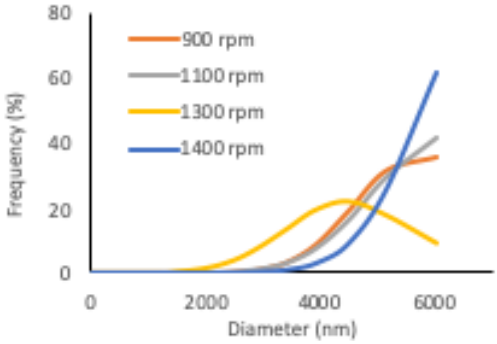


Figure 5. Effect of stirring speed on particle size of $CrPO_4$ colloids

Figure 5. shows the effect of stirring speed on particle size of $CrPO_4$ colloids. It can be seen that stirring speed between 900 and 1400 rpm, resulted $CrPO_4$ colloids with particles size higher than 2000 nm. This particle size still much larger compared to the desired one (100 - 600 nm).

The results of stirring speed optimization which was analyzed using PSA in order to see the morphology of the structure of $CrPO_4$ shown in Table 1. The results of $CrPO_4$ powder analysis with stirring speeds of 900, 1100, 1300 and 1400 rpm using PSA as shown below:

Table 1. Effect of Stirring Speed on Morphology of the structure of $CrPO_4$

Results	stirring speed (rpm)			
	900	1100	1300	1400
Median (nm)	4818.0	4932.2	3867.3	5275.0
Mean (nm)	4756.7	4831.1	3853.6	5142.3
Mode(nm)	5170.7	5242.7	4156.8	5365.3
Geo. Mean (dg) (nm)	4694.3	4766.7	3726.3	5105.6
Geo. Variance (nm)	5266.0	5411.2	13576.0	2894.1
Geo. SD (σg) (nm)	72.6	73.6	116.5	53.8

4. Discussion

The success of chromic ^{32}phosphat synthesis process is determined by the radiochemical purity of the radioisotopes ^{32}P, condensation, and temperature at the time of synthesis, pH and stirring speed. Stirring speed has to be higher than 500 rpm. Stirring speed influences the formation of colloidal particle size[10]. Therefore, in order to obtain $Cr^{32}PO_4$ colloids with the desired particle size, stirring speed during formation of $Cr^{32}PO_4$ colloids had been optimized.

Formation of $Cr^{32}PO_4$ colloids is based on oxidation-reduction shown in Equation 1.

$2CrO_4 + 3\ Na_2SO_3 + 2\ H_3{}^{32}PO_4 \rightarrow 2\ Cr^{32}PO_4 + 3\ Na_2SO_4 + 5\ H_2O$ …..(Eq.1)[9]

Based on Eq. 1 sodium sulfite will reduce CrO_3^{2-} [Cr(VI)] to Cr^{3+}, by heating with sulfite ion, Cr^{3+} will form an aquo complex ion hexaaquachromium (III) $[Cr(H_2O)_6]^{3+}$ at low pH. However, when the pH increases due to the addition of aquo sodium sulfite, complex ion $Cr(H_2O)_6^{3+}$ immediately undergoes polymerization through an olation process. In this process metal ions (Cr) form a polymer in an aqueous solution to produce a hydroxide complex which settles as a large complex hydrate in the form of $Cr(OH)_3 x\ H_2O$. Heating and high pH will incorporate phosphate ion (PO_4^{3-}) into the complex hydroxide to replace the water molecules and form insoluble compounds $CrPO_4$ (light blue)[10]. To prevent the occurrence of large aggregates during polymer formation gelatine is used as the medium so that the chromic complex will be bound by coordination to the amino acids of proteins and phosphate ions strongly bound to complex chromic. Gelatine as a protective colloid-forming a layer around colloidal particles so that the colloidal particles do not agglomerate or separate from the medium.

The particle size is greatly affected by the formation of aggregates during the process of reduction with the addition of sodium sulfite. Sodium sulfite will reduce Cr^{6+} to Cr^{3+}, while Na^+ ions will neutralize the charge of the colloidal particles thus coagulated colloid, with rapid stirring and preventing coagulation of colloidal particles can be dispersed in the solution.

Table 1. shows that stirring speed of 1300 rpm gave the finer particle size with an average particle size of 3853.6 nm and (dg) of 3726.3 nm. Whereas at the stirring speed of 1400 rpm the particle size is more uniform, indicated by the geometric standard deviation (σg) value of 53.8 nm (5142.3 ± 53.8 nm). The greater the value of σg, the lower the uniformity of size. The measured colloidal particles are all poly-disperse because the value of σg> 1.25 nm, colloidal particles are well dispersed when measuring 0.1 - 10 nm[11]. Based on the stirring speed optimization results, stirring speed of 1300 rpm can be considered to give a fairly good result in term of particle diameter size of $CrPO_4$ powders compared to other stirring speeds. In regard to its structure, this $CrPO_4$ powders might be suitable to be used as an active ingredient for making of $Cr^{32}PO4$ skin patches.

5. Conclusion

$Cr^{32}PO_4$ colloids the precursor of ^{32}P radiolabeled skin patches, has been prepared to give radiolabeling efficiency as well of radiochemical purity of 97.8%. However, the resulted $Cr^{32}PO_4$ colloids are still lacking in term of their particle size. The prepared $Cr^{32}PO_4$ colloids particle size is > 1 μm while the desired particle size <1μm. From the optimization of stirring was not too significant to affect particle size. Further optimization of the oxidation-reduction reaction.

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