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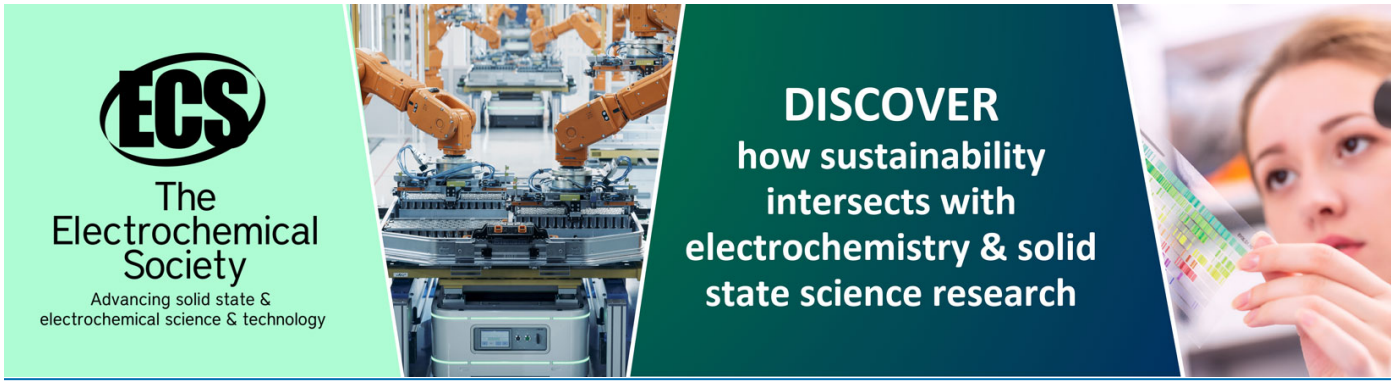

LABELED OF IRRADIATED CHITOSAN WITH IODIUM-131 RADIOISOTOPE

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Abstract. Chitosan is a polysaccharide from the deacetylation process of chitin, a compound derived from crustacean animal exoskeletons. Chitosan has benefits in various fields, one of them is in health field. Chitosan is known to be able to bind with fat so that it can reduce cholesterol levels in the blood, besides chitosan also has activities as an antibacterial and anticancer. Irradiated chitosan is carried out to degrade chitosan to smaller molecular weight and is easily absorbed to the body. As a new drug, many stages of testing are carried out to determine the mechanism and effectiveness of chitosan compound specifically as an anticancer compound. One of the required data is preclinical study data using animal model. Nuclear techniques offers the results of preclinical study that can identify quickly and accurately the distribution of a drug in the body with the radiotracer principle. Therefore it is necessary to make ^{131}I-chitosan labeled compounds irradiation (^{131}I-chitosan-75kGy) and compared with ^{131}I-chitosan labeled compounds non-radiation (^{131}I-chitosan) as controls. In this study, the optimum formulation of chitosan and chitosan-75kGy with radioisotope iodine-131 with radiochemical purity > 90% was obtained. Chitosan can be labeled by iodine-131 with the formulation of 150 μL (0.5%) chitosan, 20 μl (5 mg / mL) chloramine T, 20 μl (10 mg / mL) sodium metabisulphite, 10 μL Na^{131}I and 5 minutes incubation time at room temperature. While the optimum formulation of chitosan-75kGy labeled is 100 μL (0.5%) chitosan-75kGy, 10 μl (5 mg / mL) chloramine T, 10 μl (10 mg / mL) sodium metabisulphite, 10 μL Na^{131}I with 1 minute incubation time at room temperature. Radiochemical purity testing was carried out using ascending paper chromatography method with whatman 1 paper as a stationary phase. To separate impurities in the form of I_2, chloroform is used as the mobile phase, whereas methanol 90% is used to separate impurities in the form of I. With this successful in producing labeled chitosan, it is expected that the effectiveness of irradiated chitosan as anticancer compounds can be identified. **Keywords**: ^{131}I-chitosan, labeled compounds, irradiated

1. INTRODUCTION

Chitosan is a natural biopolymer prepared from chitin (Fig 1), which is harvested from crab and shrimp shells. The wide waters area in Indonesia causes Indonesia have the potential to produce chitin. Chitosan has been widely used for multiple applications because most abundant, low cost, renewable,

non-toxic, non-immunogenic, biodegradable and biocompatible [1]. Previous research show that chitosan have several biological activities such as antimicrobial, hypocholesterolemic agents, hepatic fibrosis, blood anticoagulant and anticancer [2–10].

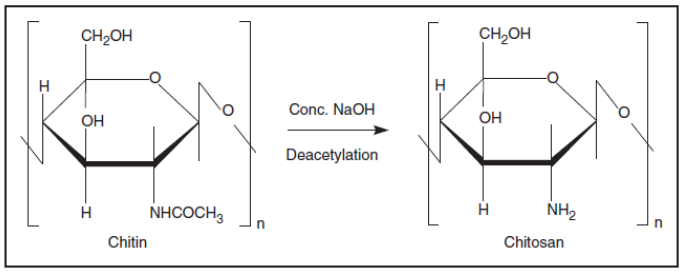

Figure 1. Chemical structure of Chitin and Chitosan [10]

Chitosan is a polymer so that solubility is low, chitosan is known to be easy to dissolved under acid solution, but in clinical applications required chitosan that can dissolve at neutral pH. Therefore, chitosan modification is very important to increase its biodegradability in human body. In addition, the biological activity of chitosan is influenced by the molecular weight. One way to accomplish this problem is to decrease chitosan molecular weight. This is generally done by enzymatic reactions. However, the weakness of this enzymatic reaction is that the process is relatively difficult and the price of the enzyme is expensive [11]. Gamma irradiation is the most popular method used for sterilization in the field of health and food preservation. Besides gamma irradiation can also be used to crosslinking and degrade polymers [12]. The advantages of gamma radiation are that the process is relatively fast, there is no residue, and the irradiation dose can be adjusted as needed. In this study, the chitosan used was 75 kGy irradiated chitosan produce by PAIR-BATAN.

Chitosan as a new anticancer agent, its effectiveness is not yet been fully established due to the lack of scientific information. A radiotracer can be defined as a specific radiolabeled molecule that monitors the in vivo behaviour of a functional molecule, and can be used to provide biological information in a living system. Hence, to provide pharmacological information of chitosan irradiated (75 kGy) for cancer treatment, we synthesized ^{131}I-chitosan as radiotracer. The aim of this study is to develop ^{131}I-chitosan under varying conditions of chitosan quantity, oxidizing agent concentration and incubation time. The resulting ^{131}I-chitosan labeled compound are then used as a radiotracer to determine the mechanism and accumulation of these chitosan in animal models in the subsequent studies.

2. MATERIALS AND METHODS

2.1 Materials

Materials that used in this research were chitosan (PAIR-BATAN), chloramine-T hydrate (Sigma Aldrich, America), sodium metabisulphite (Merck, Germany), methanol lichrosolv (Merck, Germany), chloroform (Merck, Germany), Na^{131}I (PRSG-BATAN), acetic acid (Merck, Germany), sodium acetate (Merck, Germany), sterile aquabidest (IPHA, Indonesia), pH indicator (Merck, Germany), and whattman 1 (Whattman).

The equipments that used in this experiment were dose calibrator (Victoreen), vortex mixer, single channel analyzer (Ortec), electrophoresis, and paper chromatography apparatus.

2.2 Methods

2.2.1 Labeling of ^{131}I-chitosan-0kGy / ^{131}I-chitosan-75kGy

Labeling of chitosan with radioisotope iodine-131 was performed using direct labeling method using chloramine-T as an oxidizing agent. Some parameters were varying to obtain the optimum conditions such as the amount of chloramine-T, amount of chitosan, and the incubation time. The labeling process was carried out by adding of chloramine-T solution (5 mg/1 mL acetate buffer pH 7) into the vial that contains of chitosan solution in acetate buffer pH 7 and Na^{131}I, then incubated in room temperature. After incubation time reached, sodium metabisulphite in acetate buffer pH 7 was added.

2.2.2 Optimization of chitosan-0kGy / chitosan-75kGy

To determine the optimal amount of ligand 100- 150 μL of chitosan (5 %) was used, volume was adjusted until 150 μl (to get same volume) using acetate buffer pH 7, added 10 µL of Na^{131}I (100 μCi) solution and 10 μl (5 mg/1 mL) of chloramine-T. After stirred for 60 seconds, into each vial added 10 μl (10 mg/1 mL)) of sodium metabisulphite in acetate buffer pH 7. The optimum amount of chitosan was determined from the radiochemical purity of ^{131}I-chitosan-0kGy / ^{131}I-chitosan-75kGy using paper chromatography method.

2.2.3 Optimization of Chloramine-T

Into three vials containing optimal amount of chitosan ad volume with acetate buffer pH 7 until 150 µL and add 10 µL of Na^{131}I (100 μCi) solution. Then, a varying amount (5, 10, 15, 20, and 25 µL) of chloramine-T acetate buffer pH 7 (5 mg/1 mL) was added, stirred for 60 seconds and added a varying amount (5, 10, 15, 20, and 25 µL) μl of sodium metabisulphite in acetate buffer pH 7 (10 mg/ 1 mL). The optimum amount of oxidizing agent was determined from the radiochemical purity of ^{131}I-chitosan-0kGy / ^{131}I-chitosan-75kGy using paper chromatography method.

2.2.4 Optimization of incubation time

Into four vials containing optimum amount of chitosan were added 10 µL of Na^{131}I (100 μCi) solution and optimum amount of chloramine-T solution. The solution were stirred with varying time (30, 60, 120, 150 and 300 second) in room temperature and then added optimum amount of sodium metabisulphite in acetate buffer pH 7. The optimum incubation time is the time needed to provide a high radiochemical purity of ^{131}I-chitosan and was determined using paper chromatography method.

2.2.5 Determination of Radiochemical Purity (RCP) of ^{131}I-chitosan

The determination of RCP of ^{131}I-chitosan was done using ascending paper chromatography method with whattman 1 (10 × 1 cm) as the stationary phase and methanol 90% as the mobile phase to separate the impurities of ^{131}I- and chloroform as the mobile phase to separate I_2 impurities. The chromatograms were dried in the oven at 80 oC, and then every 1 cm piece of paper was cut and measured using Single Channel Analayzer (SCA) with detector NaI(Tl).

3. RESULTS AND DISCUSSIONS

^{131}I-chitosan has been successfully labeled with iodine-131 radioisotope with chloramine T as oxidizing agent. Three parameters, chloramine-T amount, ligand (chitosan) amount and reaction time, have been varied to find optimum labeled condition. Differences in physical appearance between chitosan non irradiated (chitosan-0kGy) and chitosan irradiated (chitosan-75kGy) are chitosan-0kGy in the form of white crystals while chitosan-75kGy in brownish white. Both of these chitosan are not soluble in water, so to dissolve need to add acetic acid and adjusted to pH 7 with sodium acetate. In this reaction, the fresh chloramine-T and sodium metabisulphite dissolved in buffer acetate to prevent changes in pH that will affect the solubility of chitosan.

The influence chitosan amount in the labeling process was shown in Fig. 2 and Fig 3. Fig. 2 shows that RCP of ^{131}I-chitosan-0kGy increases with increasing amount of chitosan-0kGy. While in Fig. 3 shows that RCP of ^{131}I-chitosan-75kGy decrease with increasing amount of chitosan-75kGy. This shows that irradiation process affects the chemical structure of chitosan compounds. The main effect irradiation process will degradation of chitosan to low molecular weight [13,14]. Radiation will induced breaking of 1-4 glycosidic bonds of chitosan chain [15]. This degradation causes the chitosan-75kGy have a larger surface area than the chitosan-0kGy, so that the amount of chitosan-75kGy needed in the reaction is less than the chitosan-0kGy chitosan.

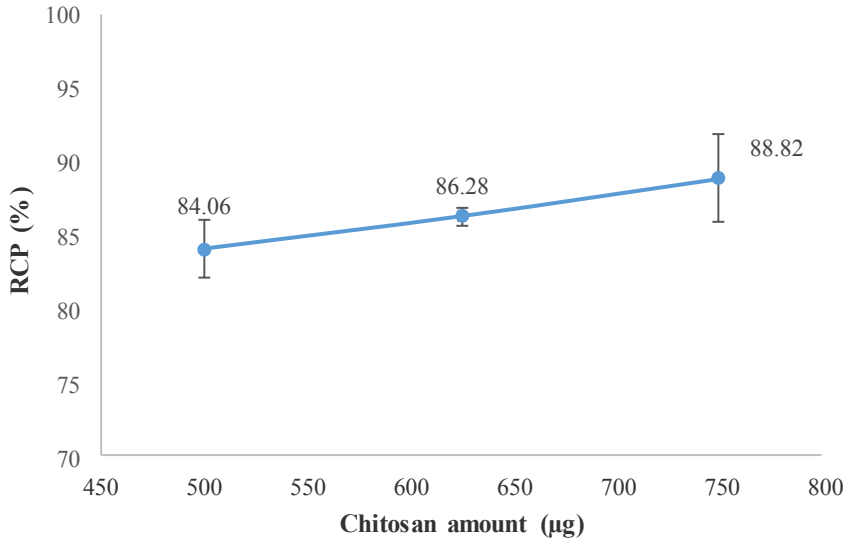

Figure 2. Effect of chitosan-0kGy amount on the RCP of ^{131}I-chitosan-0kGy

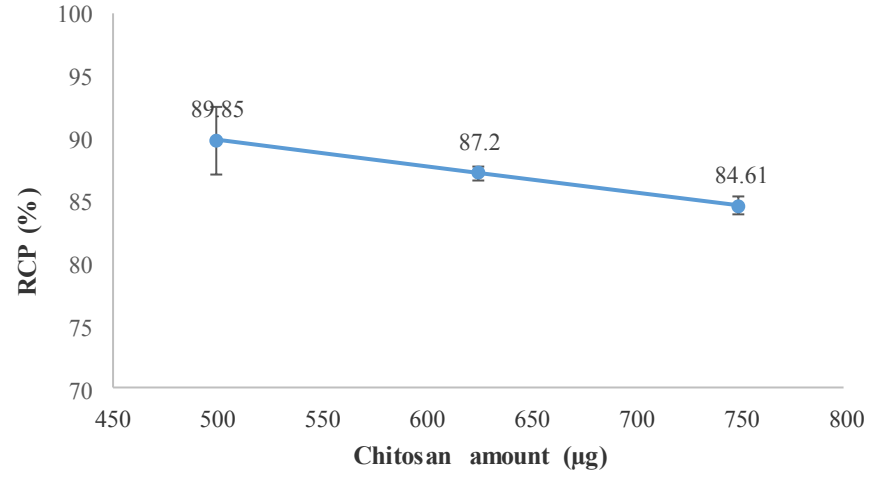

Figure 3. Effect of chitosan-75kGy amount on the RCP of ^{131}I-chitosan-75kGy

As an oxidizing agent, chloramine-T amount will influential on radiochemical purity of ^{131}I-chitosan. The powerful of chloramine-T as oxidizing agent must be controlled because may damage the chitosan compounds [16]. In this study, oxidation reaction will be shut down with added sodium

methabisulphite, the sodium metabisulphite concentration that used in this reaction is twice of chloramine T concentration. To determine influence of oxidizing amount, varying of chloramine-T amount has been examined Fig 4 and Fig 5. The chart show that labeled reaction with chitosan-0kGy optimum with 100, while chitosan-75kGy optimum with 50 μg chloramine T amount. This shows that the chitosan-0kGy reaction requires more oxidizing agents than the chitosan-75kGy. This is appropriate because the labeled reaction of chitosan-0kGy requires a greater number of chitosan amount than chitosan-75kGy reaction, so that the oxidizer agent needed in this reaction is more.

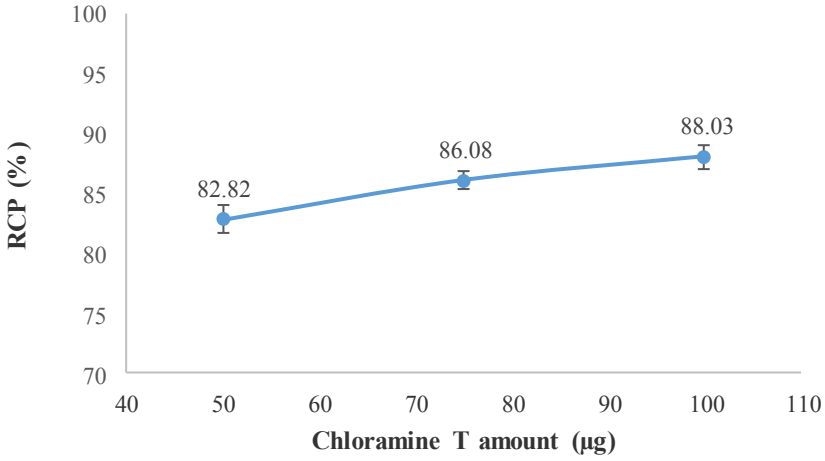

Figure 4. Effect of chloramine T amount on the RCP of ^{131}I-chitosan-0kGy

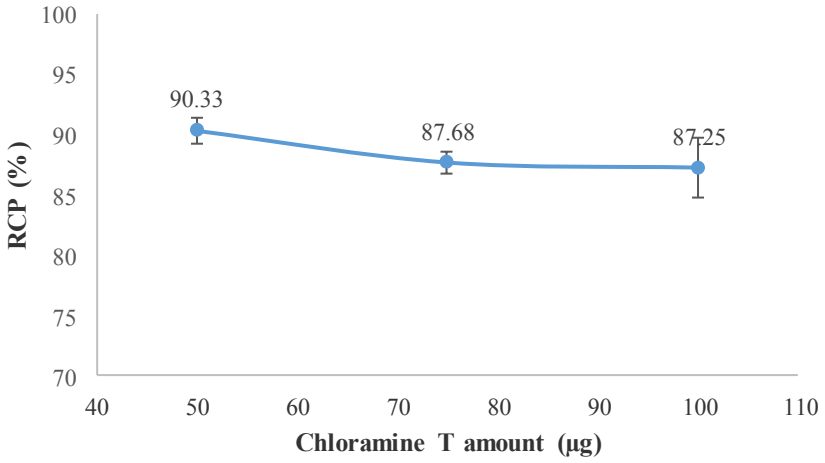

Figure 5. Effect of chloramine T amount on the RCP of ^{131}I-chitosan-75kGy

The optimum reaction time is reaction time that have the high RCP. The term reaction time in this study is the time interval between the addition of chloramine T with the addition of sodium metabisulphite. The optimum reaction time needs to be determined because if the reaction time is too short, the reaction will not be over whereas if the reaction time is too long, there will be an excessive

oxidation reaction so the radiochemical impurity will be large. In this study are not done varying reaction temperature, variations of reaction time were carried out at room temperature. The result of varying reaction time show ini Fig. 6 and Fig 7. This chart shows that time reaction do not significantly affect to RCP of ^{131}I-chitosan-0kGy / ^{131}I-chitosan-75kGy. The RCP value from 30-300 seconds is similar and 300 second was selected as an optimum ligand amount because the smallest SD value.

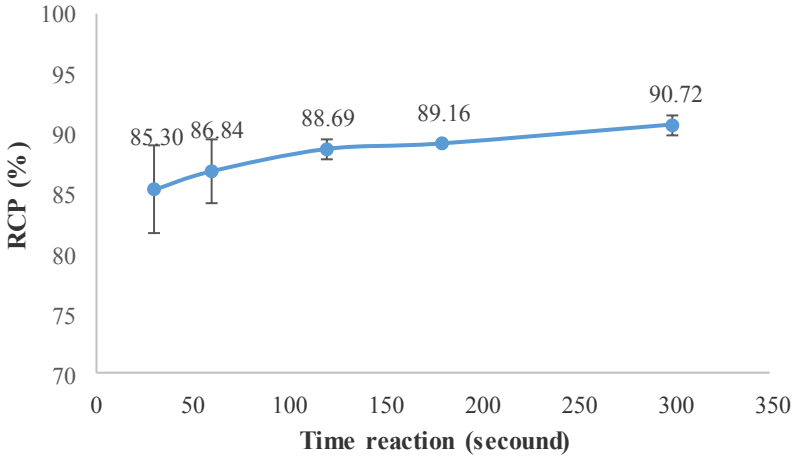

Figure 6. Effect of reaction time on the RCP of ^{131}I-chitosan-0kGy

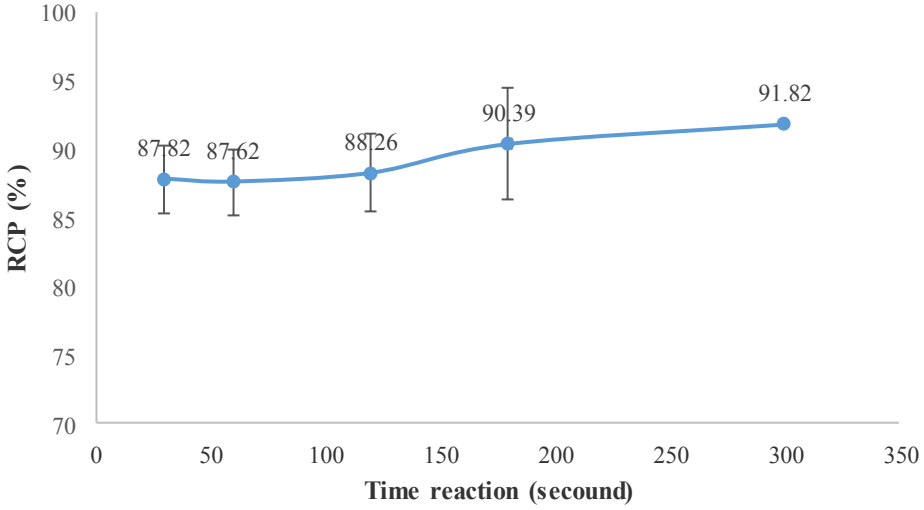

Figure 7. Effect of reaction time on the RCP of ^{131}I-chitosan-75kGy

4. CONCLUSION

The use of nuclear technology is one of the technological solutions used in acceleration the time of drug discovery and drug development research. This breakthrough is done by changing the drug compounds to labeled compounds function as radiotracer. Chitosan was labeled successfully using iodine-131 radioisotope with radiochemical purity > 90 %. The optimum labeling conditions of ^{131}I-chitosan-0kGy are 750 μg of chitosan-0kGy, 100 μg of chloramine T, 200 μg of sodium metabisulphite and 300 second of reaction time. While the optimum labeling conditions of ^{131}I-chitosan-75kGy are 500 μg of chitosan-75kGy, 50 μg of chloramine T, 100 μg of sodium metabisulphite and 300 second of reaction time. With this successfully to preparation of ^{131}I-chitosan-0kGy / ^{131}I-chitosan-75kGy, there is an opportunity to continue to in-vivo study to obtain the detailed information concerning the organ distribution.

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