

Invitro Micro Encapsulation of Beta Tri Calcium Phosphate from *Anadara granosa* Shell Synthesis

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ABSTRACT

Background: Calcium is a material that is mostly contained in the *Anadara-granosa* shell. Beta-TCP can be obtained from the hydrothermal process from the *Anadara-granosa* shell. Beta-TCP has a chemical composition that approximates the structure of bones and teeth. **Objective:** The microencapsulation technique aims to increase stability, reduce side effects and toxic effects of drugs, and prolong the release of ingredients. The encapsulation process is an attempt to inhibit the dissolution speed of Calcium to prevent tunnel defects. **Methods:** *Anadara-granosa* shell powder was subjected to hydrothermal processing for 18 hours and sintering for 3 hours. The beta-TCP powder was dissolved with aquadest using a magnetic stirrer until it was homogeneous, Na-alginate was dissolved in aquadest until it was homogeneous with a magnetic stirrer then the two solutions were mixed and the CaCl_2 solution was dropped. The sample was divided into 3 groups; Pure Beta-TCP(K-); 7 hours stirring (P1); 8 hours stirring (P2). After completion of the stirrer, the samples were centrifuged at 2500 rpm for 6 minutes, then freeze-dried for 12 hours. The level test was carried out using complexometry comparing the pure Beta-TCP group with the Beta-TCP stirrer encapsulation process for 7 hours and 8 hours. **Results:** The data showed that the average calcium level in K(-) group with pure Beta-TCP was 8.63%, the P1 Beta-TCP group with 7 hours stirrer 2.86%, and the P2 Beta-TCP group with 8 hours stirrer 2.12%. **Conclusion:** In the *Anadara-granosa* shell nanoencapsulation process, the calcium level gradually decreased with the longer duration of stirring time.

Keywords: Encapsulated, Calcium Level, Natrium Alginate Polymer, *Anadara granosa*

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INTRODUCTION

Indonesia has abundant land and sea natural resources. One of the natural resources that can be used as an alternative medicine/dental material is the shell of *Anadara granosa* (AG) which contains 98.68% Calcium Carbonate (CaCO_3). *Anadara granosa* (AG) shell can be synthesized by hydrothermal method for 18 hours at 200°C and sintering for 3 hours at 900°C to obtain hydroxyapatite (15%), Beta-tricalcium phosphate (B-TCP) (79%), and $\text{Ca}(\text{OH})_2$ (6%).^{1,2} Beta-Tricalcium Phosphate (β -TCP) ($\beta\text{-Ca}_3(\text{PO}_4)_2$) has a chemical composition that approximates the structure of bones and teeth.^{3,4} β -TCP can be used as a potential bone substitute because it is biocompatible, bioresorbable, and has osteoconductivity.^{4,5} TCP (Tricalcium Phosphate) is a material that has osteoconductive properties but has a faster resorption rate than hydroxyapatite.⁶ TCP (Tricalcium Phosphate) is also a porous bioceramic material that has non-reactivity and resorbability biological properties. Theoretically, the biocompatibility of TCP combined with calcium allows TCP to stimulate odontoblasts, thereby leading to the formation of dentin bridges. Because of it, Beta-TCP from the synthesis of *Anadara granosa* shell (AG) can be used as an alternative ingredient in dental care to maintain vitality and stimulate the pulp healing process.²

The material commonly used today is calcium hydroxide ($\text{Ca}(\text{OH})_2$) which is the gold standard material. This material has a disadvantage in long-term use, its bond with dentin tends to be mild, crushed, and dissolved in the dentin fluid resulting in a tunnel defect in the formation of the dentin bridge which can facilitate the entry of bacteria, also because of the very high pH of 11-13 this can cause necrosis of the pulp tissue.⁷

One of the technological advancements that are currently being developed is the drug delivery system in the form of microparticles.^{8,9} Microparticles can be defined as something small with a size of 1-1000 μm .^{9,10} Microparticles

formation aims to overcome the solubility of active substances that are difficult to dissolve, improve poor bioavailability, modify drug delivery systems, increase the stability of active substances and improve absorption. The advantage of microparticles is the ability to penetrate the spaces between cells that can be penetrated by colloidal particles.¹² Another advantage is the increased affinity of the system due to the increase in contact surface area by an equal amount.

Mechanical factors in the homogenization process can cause particle acceleration, vibration, emission pressure, and friction which are used to reduce the size of a particle and keep the particles small against the influence of particle growth and agglomeration.¹² Mechanical effects can be generated through rotation, magnetic stirrer vibrations, and the movement of aerated air bubbles (low energy technique), as well as from ultrasonic wave vibrations (ultrasonicators / high energy techniques).¹³ The small particle size is greatly influenced by the particle acceleration, vibration, emission pressure, and fast friction of the formulation technique used.¹⁴ Researcher wanted to add to the magnetic stirrer process when carrying out the ionic glaciation process to see the particle size and calcium levels generated from the process and compare with the material without encapsulation

MATERIAL AND METHOD

The design of this study was the post-test-only control group design to determine the application of Beta - tricalcium phosphate (β -TCP) from *Anadara granosa* shell in the drug release process. The groups were divided into 3, which was the negative control group pure Beta - tricalcium phosphate (β -TCP) was K(-), the treatment group with 7 hours microencapsulated Beta - tricalcium phosphate (β -TCP) was P1 and 8 hours microencapsulated Beta - tricalcium phosphate (β -TCP) was P2.

Anadara granosa shells are obtained from Kenjeran coastal waters, Surabaya. The



shells of *Anadara granosa* are boiled for 30 minutes. Then, brush the blood clam shells on the outside and inside using water and soap without bleach, then dried at room temperature. After that, it was crushed using a mortar and pestle and was sieved with 200 mesh to get smaller particles.

10 grams of *Anadara granosa* shell powder was added to 100 ml of aquadest to produce 1M and 6.9 grams of $\text{NH}_4\text{H}_2\text{PO}_4$ solution added with 100 ml of aquadest to produce 0.6M. Then the two were mixed with a magnetic stirrer for 30 minutes. Furthermore, the mixed solution was transferred to the reactor. The reactor was put in an electric oven to be heated to a temperature of 200 ° C for 18 hours. The result obtained was cooled at room temperature. Furthermore, the heating powder was washed with aquadest using a magnetic stirrer. Abstersion was carried out repeatedly until the reaction results were separated with distilled water, indicated by the pH to be 7. This action was done to remove acidic byproducts. Next abstersion was carried out with methanol to limit agglomeration of HA particles during drying. The samples were dried in an electric oven at 50 ° C for 3 hours and 4 hours. Sintering the sample at 900 ° C for 3 hours to remove impurities and increase the crystallinity of the sample to produce the active ingredient TCP.

β-TCP encapsulation process method

β-TCP was mixed with sodium alginate with a mass ratio for example β-TCP / sodium alginate which is 1/1. 2 gr, 2.5 g, and 3 g β-TCP powders were mixed with 2%, 2.5%, and 3% sodium alginate, and a solution was made with 100 ml aquadest for each sample. 16.65 g of CaCl_2 was dissolved with 100 ml of aquadest. Then the CaCl_2 solution was dropped into a solution of β-TCP and sodium alginate which was stirred for 7 hours for the P1 group and 8 hours for the P2 group. After that, centrifuged at 2500 rpm for 6 minutes and the sediment was taken. Then dried using the freeze-dry technique with 5% humidity. The freezing process was

carried out by storing the sample in the freezer overnight to form ice crystals, then the drying process used a freeze dryer.

Particle size analysis

Measurement of particles or PSA beta-TCP which is encapsulated aims to determine the particle size. 1 gram of BTCP encapsulated powder was dissolved in 8 ml of distilled water and sonicated for 10 minutes to obtain a homogeneous sample. The sample is placed in a suitable single-use plastic cuvette. Samples were measured with a beta nanoparticle analyzer 5 times per group. The optimal attenuator gap width is 6 to 8. Overcast samples will appear 6 in the attenuator, which means that the sample needs to be dissolved. A sample that is too transparent will show 8, meaning that more sample material needs to be added.

Calcium Rate Analysis

Calcium rate analysis using the complexometric method. The sample was weighed 0.5 g. Then dissolved using distilled water using a measuring flask until the meniscus limit showed the number 100 ml. Furthermore, 2 ml of NaOH 8N was added. and the Calcon indicator. After that, the titration was carried out using a 0.1 N EDTA reagent until it changed color from orange to blue. The final result, record the volume of the EDTA titrant 0.1 N used.

RESULT

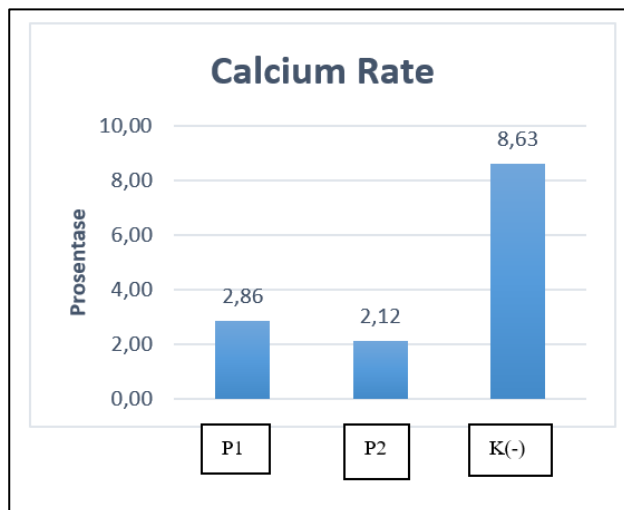
This study aimed to determine the differences in the content of calcium levels contained in β-TCP (β-Tri Calcium Phosphate) which was carried out by the encapsulation process using sodium alginate polymer. This study used a calcium level test with the complexometric method. The data generated from this study were:

Table 4.1 The results of analysis of pure calcium β-TCP levels, β-TCP encapsulation using the complexometry method

Sample	7 hours microencap sulated Beta Tricalcium phosphate	8hours microencap sulated Beta Tricalcium phosphate	Pure Beta Tricalciu m phospha te
1	2,91	2,05	8,61
2	2,76	2,14	8,50
3	2,91	2,18	8,78

Table 4.2 The results of the average analysis of pure calcium β -TCP levels, β -TCP encapsulation using the Complexometric method

7 hours microencapsulat ed Beta Tricalcium phosphate	8 hours microencapsul ated Beta Tricalcium phosphate	B-TCP 18 hours
2,86	2,12	8.63



Graphic 4.1 Calcium Rate

DISCUSSION

This study aimed to determine the differences in the content of calcium content of *Anadara granosa* shells which were carried out by the 18-hour hydrothermal process and 3 hours of sintering, which was then carried out by the β -TCP (β -Tri Calcium Phosphate)

encapsulation process with sodium alginate polymer for 7 hours and 8 hours, pure β -TCP (β -Tri Calcium Phosphate).

In this study, the results of the calcium level test showed that the average calcium level in the 7-hour micro-encapsulation group β -TCP (β -Tri Calcium Phosphate) was 2.86% greater than β -TCP (β -Tri Calcium Phosphate) 8 hours by 2.12%. Meanwhile, in the pure β -TCP group the calcium content was 8.63%. Based on the results of this study, it was explained that microencapsulation was a way of using a relatively thin coating to protect the core material from being melt so that it was easy to handle and could protect the loss of core material and as a controlled release of drugs that entered the body so that the dissolution of the core material could be controlled.^{9,10}

Microencapsulation is the process of using a relatively thin coating on small particles of solids, where the particle size ranges from 2-5000 μm . Microencapsulation or what is called a microcapsule is defined as a particle containing an active substance or core material surrounded by a coating or shell, with the presence of this polymer wall layer, the core substance will be protected from the influence of the outside environment. Microencapsulation techniques are commonly used to increase stability, reduce side effects and toxic effects of drugs, and prolong drug release.¹⁵

Microencapsulation consists of three main ingredients, that are core material, coating material, and solvent. The core material is the specific material to be coated, the core material should not dissolve or react with the coating material and solvent used. The coating material is a material that coats the core to cover unpleasant odors and tastes, protecting the environment, stability, and preventing evaporation.^{15,16}

The coating material must be able to provide a cohesive thin layer with the core material, can mix chemically, does not react with the core (is inert), and has properties suitable for the coating.¹⁶ The coating material or polymer used is alginate, which is a very

good polymer in helping microencapsulation performance. Sodium alginate produces a more uniform gel structure which forms a stronger cross-linking structure and more trapped loading of the material.^{15,17}

A simple way of making microparticles is the ionic gelation method which this method involves a cross-linking process between the polymer Na-alginate and CaCl_2 as a crosslinking ion. *Anadara* shells (AG) powder is micro-prepared and coating is carried out.¹⁶ Ionic gelation method functions as a hardener and maintains the shape of the microparticles.^{9,16} A widely used cross-linking agent is calcium chloride (CaCl_2), which this cross-linking agent can control the release of the active ingredient from the oral dosage form with alginate crosslinking.⁹

The nature of alginate gel formation with polyvalent ions such as calcium leads to the formation of cross-links. The addition of CaCl_2 to the alginate filtrate causes calcium alginate deposits in the form of white fibers and a large coarse size because the mixed Ca^+ ions will bind to the alginate and will form cross-links between molecules and then settle.¹⁹ The formation of this cross-linked bond will strengthen the mechanical strength of the particles that are formed.¹¹

Factors that can affect the properties of alginate solutions are temperature, concentration, and polymer size.¹⁶ Alginate is a polymer that is often used with the ionic gelation method, because alginate produces a good shape, is biocompatible and the resulting matrix is non-toxic factors affecting stability.²⁰

Aprilia's research stated that the beta-TCP particle size resulting from the synthesis of *anadara granosa* shell with sodium alginate polymer by stirring technique for 2 hours obtained a particle size that was closest to the ideal particle size determined for pulp capping material. Calcium levels test showed that the stirrer process for 1 hour had the highest calcium levels.² In another study, it was proven that the stirring technique using a magnetic

stirrer could synthesize calcium alginate cross-links with various alginate concentrations.²⁰

However in this study, the longer duration of microencapsulation process could reduce the calcium levels contained in the active ingredient, due to the influence of the encapsulating material or polymer that was given and also the effect of the crosslinker agent used. Polymers and crosslinkers would be cross-linked with the active ingredients, so that it could affect the amount of active ingredient.¹⁰

CONCLUSION

β -TCP (β -Tri Calcium Phosphate) was produced from hydrothermal processes and sintering of *Anadara granosa* shells using the hydrothermal method for 18 hours and 3 hours of sintering. There was a difference in calcium levels which is pure β -TCP (β -Tri Calcium Phosphate) material has more calcium levels than β -TCP (β -Tri Calcium Phosphate) that encapsulated for 7 hours and 8 hours.

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