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Nanoparticle preparation and characterization of Haruan fish (*Channa Striata*) exctract contains albumin from south kalimantan with Ionic gelation method

Rahmawanty D[1*], Risa A[1], Malikhatun N[1], Prima HR[1], Nani K[1], Effionora A[2]

***Corresponding author:**

Rahmawanty D

[1]Pharmacy Department, Faculty of Mathematics and Natural Science, Universitas Lambung Mangkurat
[2]Faculty Of Pharmacy, Universitas Indonesia

Abstract

Snakehead fish (*Channa striata*) has been reported to be used for wound healing by people in South Borneo because it contains albumin. Snakehead fish extract (*Channa striata*) has hydrophillic property and poor stability. Nanoparticle technology has been started to be developed as an alternative solution to improve drug delivery profile. The purpose of this study was to determine the formulation that obtained best characterization for nanoparticle. Nanoparticles were prepared by ionic gelation method, that was prepared by doing optimize ratio between snakehead fish extract : chitosan and pH of chitosan solvent. Nanoparticles were characterized using Particle Size Analyzer for particle size and particle size distribution, measurement of entrapment efficiency, determined Zeta potential using Particle Size Analyzer, and observation of particle's morphology using Transmission Electron Microscope. The result showed that the chosen formula was formula 6 which ratio of extract : chitosan 1:2 with chitosan solvent pH 3, particle size 152.3 nm, polidispersity index 0.778, percentage of entrapment efficiency 51.3961 %, Zeta potential +35.9 mV, and round shape of particles.

Keywords: ionic gelation, nanoparticle, snakehead fish (*Channa striata*)

Introduction

Snakehead fish (*Channa striata*) is one of 17 fish found at Awang Landas fishery, Barito river, South Kalimantan [1]. Snakehead fish (*Channa striata*) inherent in the culture of the people in South Kalimantan. According to [2] Snakehead fish (*Channa striata*) containing albumin that can be used as wound healing for open wound. Based on the constituent components, Snakehead fish (*Channa striata*) extract has hydrophilic properties and low stability.
Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm [3]. The use of nanoparticles in the delivery system of active compounds from natural materials can increase absorption, maintain the stability of the active compound, control the particle size, surface properties, and the release of pharmacologically active compound to achieve a specific action so as to obtain a therapeutic effect and optimal dose regimens [4]. Nanopartikel can be prepared by ionic gelation method. Ionic gelation method is the most convenient, simple method, does not require a lot of organic solvents, and can be controlled. In the ionic gelation method, chitosan and tripolyphosphate as the materials that form the nanoparticles. Chitosan chosen as polymer because it is biocompatible, non-toxic, biodegradable and mucoadhesive [5]. While sodium tripolyphosphate has no toxic properties, can form a bond through crosslink process with chitosan, derived nanoparticles are more stable, and has the characteristics of a better membrane penetration [6].
The ratio that was used is a very critical factor and can control the size and particle size distribution of nanoparticles [7]. Cross linking reaction also depends on the degree of acidity [8], becouse ionization of chitosan and sodium tripolyphosphate will affect the electrostatic interaction [7]. Therefore, this study needs optimization process in the preparation of snakehead fish nanoparticle extract with ionic gelation method and further nanoparticles characterization.

Materials and Methods

Materials

Snakehead fish (*Channa striata*), chitosan, sodium acetate, sodium tripolyphosphate, aqua demineralisata, distilled water, glacial acetic acid, Bovine Serum Albumin, Bradford reagent.

Extraction process

Snakehead fish (*Channa striata*) is derived from South Kalimantan. The extraction is carried out according to the method performed by David et al (2010) with slight modifications. Fillet of snakehead fish (*Channa striata*) was extracted through boiling process with a

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pressure cooker for 45 minutes. Fresh fillets were weighed, put in a pressure cooker, and filled with distilled water. The ratio of distilled water and the fish fillet is 1: 1 (distilled water: snakehead fish fillet). At the end of the extraction process, fillet removed while the extract was collected, centrifuged, taken the upper (supernatant) and filtered through what man filter paper No. 1. The next step is evaporated using a water bath until thick extract obtained, and stored in the refrigerator before use [9].

Determination of total albumin extract

Sample is made with a concentration of 10,000 ppm, by dissolving 1 gram of snakehead fish (*Channa striata*) extract in 100 mL of distilled water. Then take 0, 5 mL of sample and added with 1 mL of Bradford reagent. Furthermore, the solution is homogenized by vortex mixer and incubated at room temperature for 10 minutes. The mixing result was measured using a UV-Vis spectrophotometer at a wavelength of 568 nm [10]. To calculate total of albumin protein fish extract the results of absorbance incorporated into the standard curve equation y = bx + a.

Acetate buffer (pH4) preparation

200 mg of sodium acetate powder put in 100 ml CO2-free water and stirred using a magnetic stirrer. Then do the pH 4 adjustment by adding 3 mL of acetic acid. Chitosan solution preparation 2 mg / mL chitosan powder was dissolved in a solution of acetate buffer at pH 4, and stirring using a magnetic stirrer and Ultrasonicate at 50ºC temperature for 30 minutes. Sodium tripolyphosphate solution preparation 0.5 mg / mL sodium tripolyphosphate powder dissolved in aqua demineralisata using a magnetic stirrer.

Nanoparticle preparation (optimization ratio of extract and chitosan)

The concentration ratio of chitosan and sodium tripolyphosphate is 4: 1. Snakehead fish extract (*Channa striata*) made into a variety of concentrations: 10 mg / mL, 8 m g / mL, 6 mg / mL, 4 mg / mL, 2 mg / mL, 1 mg / mL, 0.67 mg / mL, 0.5 mg / mL, and 0.4 mg / mL. Amount 0.5 mL snakehead fish extract is inserted into mikrotube and added with 0.5 mL of chitosan solution. The mixture was homogenized using a vortex mixer for 20 seconds. Then added a solution of 0.25 mL of sodium tripolyphosphate, homogenized again with a vortex mixer for 20 seconds.

Table-1. Formula

Formula	Snakehead fish extract (*Channa striata*)	Chitosan	Sodium tripolyphosphate	Ratio (extract : chitosan)
1	10 mg/ mL	2 mg/ mL	0,5 mg/ mL	5:1
2	8 mg/ mL	2 mg/ mL	0,5 mg/ mL	4:1
3	6 mg/ mL	2 mg/ mL	0,5 mg/ mL	3:1
4	4 mg/ mL	2 mg/ mL	0,5 mg/ mL	2:1
5	2 mg/ mL	2 mg/ mL	0,5 mg/ mL	1:1
6	1 mg/ mL	2 mg/ mL	0,5 mg/ mL	1:2
7	0,67 mg/ mL	2 mg/ mL	0,5 mg/ mL	1:3
8	0,5 mg/ mL	2 mg/ mL	0,5 mg/ mL	1:4
9	0,4 mg/ mL	2 mg/ mL	0,5 mg/ mL	1:5

Nano particle preparation (pH of chitosan solution optimization)

Snakehead fish extract (*Channa striata*) made into variety of pH (pH 3 and pH 4). Preparation process is the same as on optimization of the concentration ratio of extracts: chitosan.

Nanopartikel Characterization

Characterization includes particle size measurement, particle size distribution, measurement of entrapment efficiency, determination of Zeta potential, particle morphology observation, observation of clarity and pH measurement.

Results and Discussion

Snakehead fish extract

Snakehead fish extract is brownish yellow.

Determination of total albumin extract

Snakehead fish extract solution with concentration 10000 ppm contained 1.0215% total protein albumin or 10.215 mg protein albumin contained in 1 gram of snakehead fish extract. F5, F6, F7, and F8 had choosen based on visual observations and prepicitation height, with a concentration ratio of extracts: chitosan is 1: 1, 1: 2, 1: 3 and 1: 4. Selection is based on the precipitates that form at F5,

F6, F7, and F8 begins to form on day 5 and 6. Particle size below 1000 nm not easy to precipitate, so that no deposits which occur during storage several days [11-13]. On visual observation also obtained opalescent systems which are the optical properties of the colloidal system on F5, F6, F7, and F8.

Nano particle preparation (pH of chitosan solution optimization)

Based on observations of prepicitation height, F6 and F7 had choosen. F6 and F7 has a concentration ratio of extracts: chitosan is 1: 2 and 1: 3 in pH 3 and pH 4 chitosan solvent and characterized the particle size, particle size distribution, and percentage of entrapment efficiency.

Nanopartikel Characterization

Particle size and particle size distribution

Table-2. Particle size and PI value

Formula	pH	Particle size	PI
F6	3	152,3	0,778
	4	233,8	0,607
F7	3	233,15	0,859
	4	133,1	0,775

Based on the table it appears that all formulas are in nano size range, ie 1-1000 nm with a polydisperse index of more than 0.5. It shows that optimization has been done to produce particles in the nanometer size.

Entrapment Efficiency measurement

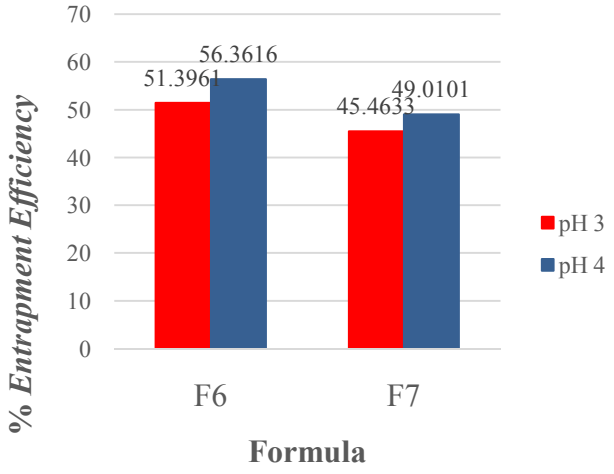

Figure-1. Entrapment Efficiency

Based on the graph shown in F6 pH 3 and pH 4 obtained percentages of entrapment efficiency 51.395% and 56.360% means that more than 50% active ingredients adsorbed into the nanoparticle system. While F7 at pH 3 and pH 4 obtained entrapment efficiency 45.461% and 49.008% means more than 40% active ingredients entrapped into nano particles. Idealy nano particle system should have high entrapment ability of active ingredient (entrapment efficiency) [14]. The results entrapment efficiency with the largest value, selected formula 6 with a concentration ratio of extracts: chitosan at 1: 2 in a solvent chitosan pH 3 and pH 4.

Zeta Potential measurement

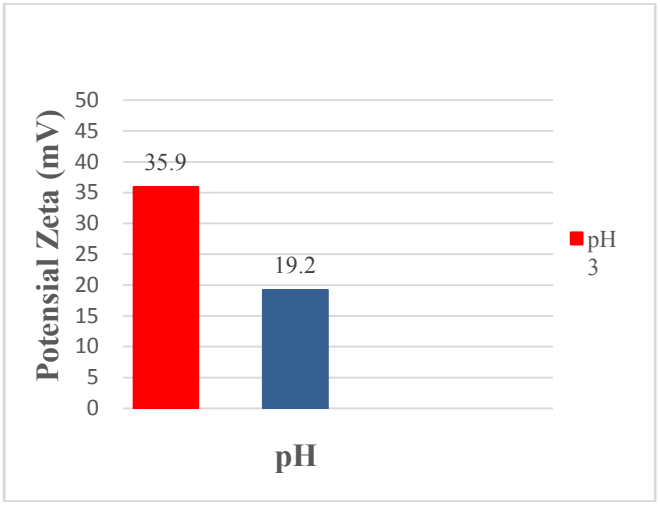

Figure-2. Zeta Potential

Based on the graph shows that the nanoparticles with the formula 6 pH 3 shows a stable colloidal system because it has a Zeta potential value of more than (+/-) 30 mV. While the formula 6 pH 4 shows the colloidal system is less stable because it is not close to the value (+/-) 30 mV. Zeta potential is generated both formulas have the charge positively charged positif. Nanopartikel that can bind to the membrane which has a negative charge, such as the cornea and intestinal cell membranes of the gastrointestinal tract, thus facilitating cellular uptake include pinositosis processes, endocytosis is not specific or receptor mediated endocytosis or phagocytosis [15].

Morfologycal observation with Tranmision Electron Microscope

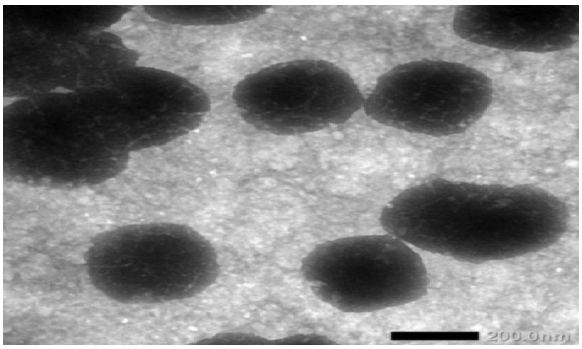

Figure-3. Morfologycal observation

Based on the pictures it appears that at pH 3 F6 has spherical shaped particles with relatively uniform size and included in the nanometer size range. Nanoparticles good thing is that has a spherical shape because it is more stable thermodynamically (Rizki, 2014). Less spherical particle shape which will facilitate contacts antarpartikel so would cause aggregation [16].

Visual Clarity Observation

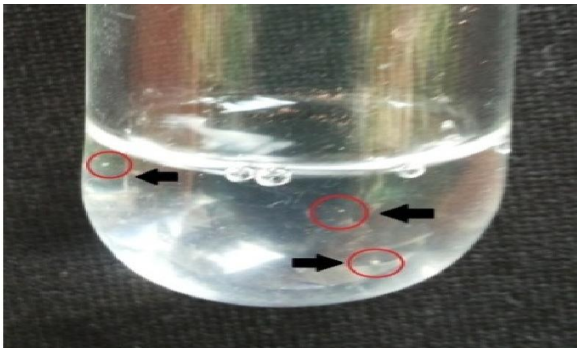

Figure-4. Visual Clarity Observation

Based on the image the system that form namely opalescent system, the existence of particles that float in the liquid phase. Ooptical properties of colloidal system known as Faraday-Tyndall effect, when the colloidal system passed by scattering light rays will occur, so that the visual observations will appear as a system opalescent [17].

pH measurement

Based on pH observations, obtained that pH value for the F6 is 33.62. The value of 3.62 indicates that the resulting nanoparticles are acidic, because the pH of the solvent used is chitosan acetate buffer pH 3.

Conclusions

Formula 6 with a concentration ratio of snakehead fish extract: chitosan with a ratio of 1: 2 in a chitosan solvent pH 3 produce the most excellent characteristics nanoparticle, which has particle size 152.3 nm, percentage of entrapment efficiency 51.3961%, and Zeta potential +35 , 9 mVand spherical morphology.

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