Formulation Nanocapsule of Turmeric Extract, Characterization on Oral Administration and Its Ability as Antibacterial

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Abstract-The use of turmeric is restricted by its low solubility in water, therefore it has low bioavailability. This obstacle can be solved by the development of nanoparticle technology. This study aimed to develop nanoparticles formulation using turmeric extract and chitosan as the matrix and sodium tripolyphosphate (STPP) as cross linker, to study its characteristics on oral administration and capabilities as antibacterial. The method used in the formulation of nanoparticles was ionic gelation followed by spray drying on formula A and oven drying at 50ºC on B. The result of nanoparticle or nanocapsule characterizations are: average size of particles on A and B is 428 nm and 840 nm, its zeta-potential on A and B is -11.47 mV and -20.22 mV, with spherical particle morphology. Entrapment efficiency on A and B is 88% and 78%.These formulas also has a good stability in AIF (Artificial Intestinal Fluid) after 3 hours on A and B is 78% and 51%. Its ability as antibacterial agent was evaluated by agar diffusion on *E. coli* bacterial, both formulas provide a middle inhibition in diameter clear zone 7-10 mm. It was concluded that A formulation of nanocapsule turmeric extract has characteristics can used oral administration and potential as antibacterial.

Index Terms- Formulation and Characterization, Nanocapsule turmeric-extract, Oral administration, Antibacterial.

1. INTRODUCTION

A wide range of antibiotics are used in poultry not only to treat disease but also to maintain health, promote growth and enhance feed efficiency [1]. The uncontrolled and unlimited use of these antibiotics may however lead to the accumulation of undesirable residues in the animals treated and their products [2]. It turns out leaving residues of antibiotics in meat (50% of provision declines with the duration) [3]. Therefore it is necessary to find a solution to replace the use of natural materials such as antibiotics. Curcumin is a yellow polyphenol chemical contained in turmeric rhizome (*Curcuma longa* L.), it has various wide spectra of biological activities. Antibacterial activity of curcumin related to polyphenol can denature protein [4] and ruin the cell membrane bacterial [5].

The wide use of curcumin for the therapy is limited because of its low solubility in water so it has a low systemic bioavailability [6]. Curcumin also easily degraded in neutral to basic pH (as intestine condition), and easily photo-degraded as well [7]. Nanoparticle is a colloidal structure with the size ranged within 10-1000 nm [8]. Nanoparticle can deliver chemicals better into the small units in the body; overcome the resistance problem caused by body's physiological barriers related to pore size factor; and its possible to be targeted, so it can reduce drug's toxicity and improve drug distribution efficiency [8]. For the cellular uptake study through fluorescence microscope, it was found that the complex has an ability to penetrate cellular membrane into the cytosol. The cytotoxicity study of nanocurcumin is non-toxic to normal cell line [9]. Chitosan is a natural biopolymer obtained by alkaline deacetylation of chitin /crab shell [10]. Chitosan has various excellent properties as biocompatible, bio-degradable, low toxic and not immunogenic. Chitosan is widely used together with TPP polyanion in various nanoparticle formulation study by ionic gelation method. Ionic gelation method is engaged to the forming of the complex by the two oppositely charged structures which then form nanoparticle gel. Ionic gelation is a very simple and easy preparation method [11]. This molecular weight takes influence in its solubility and viscosity. Short chain chitosan is dissolved easily in acidic organic solvents such acetic acid, citric acid, and tartaric acid [12]. Application of nanocurcum into livestock as well as for cancer drugs to humans is considered expensive, for it is in this study the formulations nanoparticles using turmeric extract and industrial chitosan cross linked with technical TPP were developed. Besides, the concentration of the base material and method of drying is also necessary to find an economical price.

2. MATERIALS AND METHODS

2.1. Nanoparticle formulations

In this preliminary experiment, formulation optimization of the turmeric extract nanocapsule was run by mixing solution with a concentration of turmeric extract in ethanol 96% (0.01 and 0.02% w/v) and chitosan solution in acetic buffer at pH 4 with a concentration of (0.01; 0.02 and 0.03% w/v). Turmeric extract solution with a volume of 0.5 mL was put in eppendorf tube then added with 0.5 mL chitosan solution. After the adding of chitosan solution, the mixture was homogenized using vortex for 20 seconds. Into the homogenous mixture, TPP solution with a final concentration of (0.01 and 0.02% w/v) was added and immediately homogenized again using vortex for 20 seconds. The dispersion of nanoparticles was observed to count the amount of undissolved turmeric extract for 7x24

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hours to determine the optimum mixture. The optimum formulation differented by TEM to be used for further processing scaling up. Have found the optimal formula of nanocapsule (for substitute antibiotics in broiler chickens) is comparison turmeric extract: chitosan: TPP = 0.02%:0.02%:0.01% = 2:2:1. Furthermore, to the efficiency of the production cost will be made formula A concentration of 0.2% by spray drying and the formula B concentration of 2% to the oven drying 50ºC to characterize.

2.2. Nanocapsule Characterizations

Determining the size and the zeta potential of nanocapsules was measured using particle size analyzer (PSA Delsa™ Nano Beckman Coulter). Five mL aquadest was then added to two drops of suspension 0.1% turmeric extract nanocapsules. This mixture was mixed by tossing and turning the solution. Three mL of the mixture was taken and put in a cuvette for analysis.

Observing the morphology of nanocapsules was done using transmission electron microscopy (TEM, JEM 1400). Nanocapsule powder were dissolved in distilled water with a concentration of 0.1%, then suspension was vortex and sonification until dissolved, if poorly soluble can be helped with a few drops of alcohol. Samples of nanoparticles were spilled over carbon coated copper grid and then by means of Auto Carbon Coated (JOEL JEC-560, Japan) for 5 seconds then dried at room temperature for 15 minutes. Once the nanoparticle samples were dried, they were coated again with the aforementioned carbon then copper grid was entered into the holder and samples were ready to analyze by the voltage acceleration and magnification was suitable.

Determining entrapment efficiency. The 10 mg of turmeric extract nanocapsules were weighed then dissolved in 10 mL distilled water added with 10 mL ethyl acetate. The mixture was shaken and the ethyl acetate phase was separated. Turmeric extract nanocapsules/Curcumin dissolved in ethyl acetate was then measured for the absorbance at a wavelength of 418 nm. Curcumin levels calculated by including absorbance values into the regression equation of the standard curve have been obtained previously (Y = 4.428x + 0.114, R^2=0.999) with x = absorbance (nm) and Y curcumin content (µg/µL).The entrapment efficiency was calculated with total curcumin minus free curcumin divided total curcumin multiplied by 100%.

Stability test of the nanoparticle. The 10 mg nanocapsules powder was incorporated into Erlenmeyer and added with artificial intestinal fluid (AIF) of 100 mL. AIF is a physiological salt solution containing 20 mM bicarbonate, 139 mM chloride, 5 mM potassium, 140 mM sodium, 4 mM calcium and 3 mM magnesium adjusted to pH 7.0 with acetic acid [13].The solution was shaken using Orbital Incubator Shaker (Environ Shake) at 41°C and 50 rpm. Samples were taken in hour 0, 1, 2, 3, and 4 with 3 replication for each sample. The samples were introduced into a separating funnel and were added with 5 mL of ethyl acetate. The mixture was shaken and the ethyl acetat phase was separated. The concentration of curcumin which was not encapsulated into nanoparticle and included in ethyl acetate was measured for its absorbance at wavelength of 418 nm and stability was the calculated with total curcumin minus free curcumin divided total curcumin multiplied by 100%.

2.3 Antibacterial test.

The study tested the antibacterial power of two samples powder of nanoparticles turmeric extract on *E. coli*. Samples powder of nanoparticles are formula A-221 by concentration 0.2% and formula B-221 by concentration 2% with concentration series of treated nanoparticle turmeric extract (0.0 / negative control/ethanol), created were 62.5; 125; 250, 500, 1000 and 2500, 5000 µg /mL) and positive control /kanamycin 3000 µg/mL, with each treatment by 8 replications. Nanoparticles inhibition of pathogenic bacteria (*Escherichia coli*) was measured. Activity test was carried out by diffusion method by culturing the bacteria in nutrient agar (NA) media and pasting paper disk containing nanoparticles material sample or antibiotics as much as 10µL/disk.

3. RESULTS AND DISCUSSION

3.1. Nanocapsule Formulations

The optimal formulation nanocapsule as feed additive of turmeric extract:chitosan:TPP was 0.02:0.02:0.01% (w/v) = 221 by concentration 0.02%. Because this formula had cost drying per unit weight of powder nanocapsule too expensive for applied on broiler breeding then, these concentrations were made into formula A-221 by concentration 0.2% and B-221 by concentration 2%. These formulations were scaled-up into 1000 mL and followed by spray drying on A and oven drying at 50ºC on B to turn the solution into powder. The colour of nanocapsule powder obtained ranged from orange on A (Fig 1A) to orange brownish on B (Fig 1B).

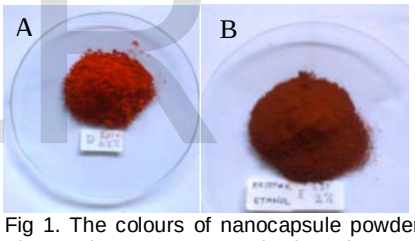

Fig 1. The colours of nanocapsule powder of turmeric extract on A and B formulas.

3.2. Nanocapsule Characterizations

Particle size and zeta potential was obtained by using *Particle Size Analyzer* at Balai Inkubasi Teknologi BPPT PUSPIPTEK Serpong, Indonesia. The observation was done at 25°C temperature with water as the solvent. From the result of the observation, the formulation of turmeric-extract nanocapsule had particle size cumulants results of 428.30±24.48 nm on A-221 by concentration 0.2% and 840.87±30.90 nm on B-221 by concentration 2% (Table 1).

Zeta potential on A-221 by concentration 0.2% and B-221 by concentration 2% is was -11.47±1.56 mV and -20.22±0.45 mV respectively (Table 1). Nanoparticle colloid has many beneficial properties namely nano-size making it sediment more stably and slowly. Particles with size less than 400 nm have good properties in drug delivery[14]. Polydispersibility index obtained from the measurement of particle size was 0.231 on A-221 by concentration 0.2% and 0.332 on B-221 by concentration 2%. Polydispersibility index describes the distribution of particle sizes present in the preparation of nanocapsule, in which the smaller the number of polydispersibility index, the more uniform the sizes of the particles any significant size difference between the larger particles and the

smaller ones will affect the particles' characteristic. The larger the particles size, the easier the particle will settle [15]. The entrapment efficiency was administered to find out the ratio between curcumin loading content that can be entrapped into nanocapsule versus total curcumin used in the formulation. The entrapment efficiency value of nanocapsule was 78 - 88% (Table 1). Accordingly, the formulation of nanoparticle formula A-221 by concentration 0.2% showed a greater entrapment efficiency value than that of formula B-221 by concentration 2%. Because at lower concentrations nanocapsule with the same amount of solvent so materials nanocapsule as turmeric extract, chitosan and TPP more soluble so that each can provide more active groups to form ionic bonds between the molecules of different charge, produces diameter size, entrapment efficiency and stability of nanoparticles greater.

TABLE 1.
CHARACTERISTICS OF NANOCAPSULES TURMERIC EXTRACT

Characteristics	Formula nanocapsule		t-test significantly
	A-221, by concentration 0,2%	B-221, by concentration 2%	
Diameter particles	428.30 ± 24.48	840.87 ± 30.90	0,0058
Zeta potensial	-11.47 ± 1.56	-20.22 ± 0.45	0,0078
Entrapment efficiency	88.29 ± 0.37	77.66 ± 1.76	0.0112

Characterization of morphology nanoparticle using *Transmission Electron Microscopy* (TEM) was performed at the Laboratory of Chemistry, Faculty of Mathematics and Natural Science, Gadjah Mada University in Indonesia. The results of formula A-221 by concentration 0.2% (Fig 2A) and B-221 by concentration 2% (Fig 1B) showed that the nanocapsule character had spherical shapes. It seemed that some nanocapsule bind together, forming larger size. This was indicated on the nanocapsule that bind together because they were not perfectly encapsulate. FTIR analysis of nanoparticle showed phosphate groups of TPP bind to the ammonium groups of chitosan nanoparticles formed and electrostatic interaction soccured between the hydroxyl groupof PGV-0 (derivate curcumin) with the amine group of chitosan [16].

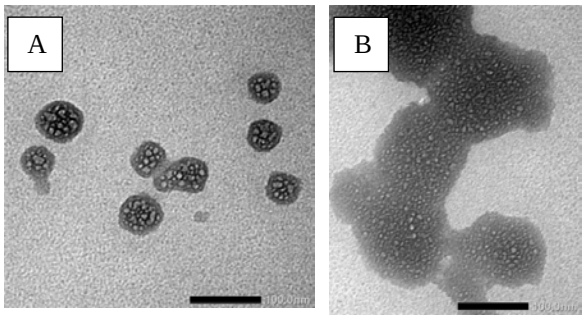

Fig 2. Transmission electron micrographs at 60000x magnification of nanocapsule turmeric extract. Image of colony Formula A-221 by concentration 0,2% (A), Formula B-221 by concentration 2% (B).

The stability of nanoparticle was tested by dissolving the nanocapsule into artificial intestinal fluid (AIF), then the amount of free curcumin solution from hour 0 to hour 4, using UV-Vis Spectrophotometer at the maximum wavelength (λ) was obtained from the scanning of the standard curve. In this experiment, the maximum wave length was at 418 nm. The release of curcumin from nanoparticle formulation was binding competition between curcumin and electrolyte with chitosan. Another factor affecting curcumin release at pH 7, NH_3^+ in chitosan was transformed into NH_3. The stability of nanoparticle showed (Table 2) that 40-80% of curcumin was still in the nanoparticle state. On the zero incubation time stability formula A (amount of curcumin is still in the capsule) greater than B significantly different ($P<0.05$) due to different concentrations of turmeric extract (curcumin), chitosan and STPP used in the manufacture with the same of the amount of solvent (1000 mL) and the differences in drying (spray drying and oven drying at 50°C) in both formulas. At lower concentrations (formula A) allows the starting material can be dissolved so have more free reactive side (negative charge of curcumin) to join the other side of the reactive molecules (positive charge of chitosan) to form ionic bonds are stronger, therefore when incubated in AIF ionic bonds at lower concentrations are not easily severed by electrolyte salts that exist in the AIF causes curcumin capsules are still bound to be much more than in larger concentrations (formula B). Similarly, the incubation period is another 1, 2, 3 and 4 hours. For the example nanocapsule stability of turmeric extract-chitosan-TPP to 3 hours in the AIF was 78.21% on A-221 concentration by 0.2% and 51.38% on B-221 by consentration 2%. The addition of TPP as a stabilizer, gave effect between cross linked chitosan (positive charge) and TPP (negative charge) by ionic interaction on the particle. Aiming if the product is administered orally in animals, chitosan is not broken all by gastric acid and proteases that curcumin bound to chitosan is still not degraded in the gut and can be absorbed into the body.

TABLE 2

STABILITY OF NANOCAPSULES TURMERIC EXTRACT ON AIF

Incubation time (hour)	Formula		t-test significantly
	A-221 by Concentration 0.2%	B-221 by concentration 2%	
0	62.442±12.78	42.482±9.48	0.014
1	66.197±5.99	51.990±5.42	0.083
2	69.037±3.20	48.083±8.00	0.075
3	78.212±0.62	51.375±5.22	0.014
4	73.097±1.44	40.073±9.55	0.020

3.3. Antibacterial activity

The range diameter of inhibition of turmeric extract nanocapsule was between 5-10 mm (Table 3). Middle category as agent antibacterial with inhibition zone of less than10 mm [17]. Becouse the zeta potential of nanocapsule has negative charge (-10) - (-20) mV (Table 1), this value is small enough to contact with the bacterial cell wall which also has negative charge [18]. The discrepancies between the results of antibacterial activity formula A-221 by concentration 0.2% with a formula B-221 by concentration 2% were possible due to the different manufacturing process in which formula A-221 by concentration 0.2%, applied spray dryer for 2 hours 15 minutes/1000mL, heated at temperatures above 120°C and below 73°C, while formula B-221 by concentration 2% applied oven heating at 50-60°C for 14 hours. The differences in temperature and length of heating caused essential oils that also act as a more violent antibacterial. Curcumin is difficult to dissolve in the water while the bacterial growth medium is water, therefore curcumin is difficult to propagate or diffuse to media to inhibit the growth of bacteria.

TABLE 3
INHIBITION ZONE OF NANOCAPSULES TURMERIC EXTRACT ON GROWTH OF *E. coli* (mm)

Concentration of Nanocapsule (µg/mL)	Formula		t-test significantly
	A-221 by Concentration 0.2%	B-221 by concentration 2%	
62,5	7.375±0.694	-	-
125	8.062±0.358	-	-
250	10.025±0.324	-	-
500**	10.112±0.368	7.287±0.633	0.000003
1000	8.275±0.341	8.063±0.358	0.021125
2500	8.087±0.318	10.025±0.324	0.000000
5000	7.287±0.633	8.087±0.318	0.012841
Kanamycin 30 µg/disk (positive control)[1]	26.125±1.808	25.875±1.642	0.356017
Ethanol 96% (negative control)	-	-	

** significantly different (P<0.01), [1] *E. coli* sensitive on kanamycin with inhibition zone ≥18 mm [19].

4. CONCLUSIONS

The optimal formula for production of nanocapsule to be applied on oral administration using turmeric extract: chitosan: STPP is formula A-221 by concentration 0.2%:0.2%:0.1% w/w, which can be developed with ionic gelation method producing nanoparticle with characterization:

1. The average particle size is 428.30 ± 24.48 nm, zeta potential -11.47 ± 1.56 mV with spherical shape.
2. Entrapment efficiency is 88.29%.
3. Stability in AIF until 3 hours is 78.21 % (good for oral administration).
4. Middle category as agent antibacterial with inhibition zone of less than10 mm.

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