The effect of centrifugation speed and Chitosan-Sodium Tripolyphosphate ratio toward the nanoencapsulation of Sambiloto (Andrographis paniculata) for the formulation of Hepatitis B drug

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Abstract. Hepatitis B is a viral infection which attack the liver. One of the compound that can overcome and inhibits Hepatitis B is Andrographolide. The compound was derived from Sambiloto plants (Andrographis paniculate). Andrographolide compound works by inhibiting α-glucosidase which assists the secretion of Hepatitis B virus. The goal of this research is to make nanoencapsulation of sambiloto leaf extracts that was encapsulated in chitosan and STPP. The nanoencapsulation will increase the bioavailability of the body for the administered Andrographolide. The size of the resulting particle at a variation of centrifugal speed of 8.000 RPM with the concentration ratio of chitosan : STPP equals to 0.2%:0.1% (g/mL), was 68.3nm. The loading capacity of the nanoparticles is 67.20% and the encapsulation efficiency of the nanoparticles is 99.48%. The release profile has a cumulative release of 34.55% with slow release in gastric pH conditions and followed by a burst release in intestine pH conditions.

1. Introduction

One of the bad habits of society Indonesia is low in maintaining healthy. A less healthy causing the incidence of various health problems, such as the emergence of a wide range of dangerous diseases that can attack all of ages, from adult to Toddler-aged children. The cause of the occurrence of many diseases is due to the inability of the body against viruses, bacteria, as well as hazardous substances that are carcinogens. One of the dangerous diseases caused by virus is Hepatitis, which is comprised of Hepatitis A, B, C, D and E. Hepatitis is a disease characterized by inflammation that occurs in the liver organ [1]. Indonesia is a country with a high additional Hepatitis B is the second largest country in the South East Asian Region (SEAR) after Myanmar [2]. There are about 2 billion people in the world infected with the Hepatitis B virus and 240 million people of whom become people with Chronic Hepatitis B [3].

World Health Organization (WHO) states that the efforts that have been made to solve the Hepatitis B sufferers is to use adrenokortikosteroid, corticosteroids, and lamivudin, but these things have not been able to effectively solve the viral infections. Hepatitis B antivirals such as drugs usage lamivudin already available and in development stage, but the drugs have not been evaluated for the treatment of acute hepatitis B [3]. One of the compounds that can inhibit even the deadly hepatitis B...
virus replication is andrographolide. The source of the andrographolide found in Sambiloto (Andrographis paniculata). One of the andrographolide activity is can inhibits the α-glucosidase enzyme \cite{4,5} which is a constituent of glikohidrolase enzymes i.e. enzymes that contribute in the formation of the viral envelope and is responsible for glikohidrolasi protein. The presence of inhibits glikohidrolase enzyme, then virus replication is not perfect and the virus will die because of the viral envelope formation will be blocked \cite{6}.

In this research, performed the extraction of sambiloto leaves with encapsulation by nano-sized particles that will be used is Chitosan. Chitosan has a fragile mechanical properties, so it must be stabilised by using sodium tripoliposphat (STPP) as a crosslinker, so when passing a very acidic pH, Chitosan is still not degraded and active substances not apart before reaching the target\cite{7}.

In this research was conducted on variation towards the concentration ratio Chitosan and STPP, because that affects the ratio of a particle may be nano-sized. Futhermore, it also conducted on variation of the centrifugation speed, to known how the appropriate centrifugation speed so that the particles that form can nano-sized. Then, controlled release by in vitro was done to know the performance of the extract of sambiloto has in nanoencapsulation as the hepatitis B drug.

2. Methods

Variations on this research is ratio of concentrations chitosan-STPP and centrifugation speed. Continued with particle distribution measurement by using particle size analyzer (PSA), determination of morphology using SEM and FTIR, quantitative test of andrographolide by using HPLC, and controlled drug release of nanoparticles in in vitro.

2.1. Extraction of Sambiloto and quantitative test of Andrographolide

Sambiloto leaves powder was dissolved in solvent ethanol 70%. Then extraction using sonicator for 50 minutes at frequency of 30 Hz and 25°C, continued by solvent evaporating using vacuum rotary evaporator. Calculating of andrographolide in sambiloto extract by using HPLC.

2.2. Nanoencapsulation

The selected method in the nanoencapsulation is ionic gelation method. Chitosan 0.2 g was dissolved in 100 mL acetic acid 1% (b/v), added 3 g of sambiloto extract, and dissolve 0.04 g 40 mL STPP into akuades then added 200 μL Tween 80 0.1% (v/v). Solution of STPP then added into chitosan-extract solution with flow speed of 0.75 ml/min accompanied with magnetic stirring for 2 hours. Then do size diminution using sonikator with 130 Watts and frequency of 30 Hz for 50 minutes and centrifugation for 15 minutes with a pause of 1 minute every 5 minutes. The last is drying using freeze dryer to a powder.

2.3. Controlled drug release

Controlled drug release was done on a synthetic fluid medium, i.e. in series in a seven hours. Synthetic fluid medium that is used in the two of kind of buffers that indicates the human digestive system, i.e. the Simulated Gastric Fluid (SGF) pH 1.2 which indicate gastric conditions and Simulated Intestinal Fluid (SIF) 7.4 pH which suggests intestinal conditions.

3. Result and discussion

3.1. Nanoencapsulation of Sambiloto extract

Method of extraction sonication produce yield 5.44% with active substance andrographolide 17.60%. The concentration ratio of Chitosan: STPP (g/mL) are made in different variations, i.e. 0.1% : 0.1% ; 0.1% : 0.15% ; 0.15% : 0.15% ; 0.1% and 0.2% : 0.1%. Continued with the variation of centrifugation speed, i.e. 8,000 rpm, 10,000 rpm, 13,000 rpm and 15,000 rpm.
3.2. Particle size distribution of Chitosan:STPP Ratio

Particle Size Analyzer (PSA) test beginning with the sample dispersion in the aquademin with the addition of Dispersing Agent Coulter Type 1A. Here are the average particle size of the variation ratio of Chitosan: STPP obtained from the PSA analysis.

| Concentration Ratio of chitosan : STPP (g/mL) | Z-Average (nm) | Particle Size Distribution (nm) |
|---------------------------------------------|----------------|----------------------------------|
| 0,1%:0,1%                                   | 1917           | 90.78 ± 12.15                    |
|                                             |                | 125.2 ± 17.38                    |
|                                             |                | 232.7 ± 32.15                    |
|                                             |                | 206.6 ±27.87                     |
| 0,1%:0,15%                                  | 2569           | 131.9 ± 18.32                    |
| 0,15%:0,1%                                  | 1454           | 144.1 ± 17.03                    |
|                                             |                | 269.2 ± 47.53                    |
|                                             |                | 62.68 ± 8.29                     |
| 0,2%:0,1%                                   | 1424           | 170.5 ± 24.99                    |
|                                             |                | 90.41 ± 12.91                    |
|                                             |                | 1212 ± 153.2                     |

Based on the table, that the higher concentration of chitosan than concentrations of STPP will earn average particle size (z-average) getting smaller. It is caused due to an increased of STPP concentration will enhance physical endurance of the particle, because of the increasing number of STPP will make ionic-crosslinking reaction by the positive force of chitosan more stable forming physically, but also increased nanoparticle diameter. Particle size distribution obtained from each variation has a relatively small size and reach a size of nanometer, but z-average or average particle size is still big even reach a size belongs to the micro. The Z-average size will only be comparable with the size measured by other techniques if the sample is monomodal, spherical or near-spherical in shape, monodisperse, and the sample is prepared in a suitable dispersant, as the Z-Average mean size can be sensitive to even small changes in the sample, e.g. the presence of a small proportion of aggregates.

3.3. Particle size distribution of Centrifugation Speed Variation

Based on the measurement of particle distribution, size obtained reach the nano is a variation of centrifugation speed 8,000 rpm i.e. 68.3 nm and 1663.3 nm at centrifugation speed 15,000 rpm. It is because at the high centrifugation speed will make particles collide and can cause clots flok. That is corresponds to the Budiyono (1999) who explains that fluid movement due to stirring will be followed by microflok formed. Here are the average particle size of the centrifugation speed variation obtained from the PSA analysis.

| Centrifugation Speed (rpm) | Z-Average (nm) | Particle Size Distribution (nm) |
|----------------------------|----------------|----------------------------------|
| 8.000                      | 68.3           | 22.4 ± 3.2                       |
|                            |                | 86.3 ± 27.3                      |
| 10.000                     | 1160.7         | 964.5 ± 161.8                    |
|                            |                | -                                |
| 13.000                     | 666.0          | 1.3 ± 0.3                        |
According to Jong (2008)\cite{11}, the size of nanoparticles used as drug carriage has a range of <100 nm, so at the centrifugation speed 8,000 rpm can be said that the particles sucessfully formed with nano size.

3.4. **Morphology determination using SEM**

Morphology of the particles observed with the Scanning Electron Microscope (SEM). The resulting morphology is not smooth and has no pores on its surface, occurs the agglomeration, and the form is not spherical.

![Figure 1. SEM result of nanoparticle](image)

3.5. **Loading capacity and encapsulation efficiency**

Loading capacity shows the number of drugs contained in nanoparticles formed in this paper, the drug was active substance andrographolide in the crude extract of sambiloto. The results of the calculation of loading capacity from each variation can be seen below:

![Figure 2.](image)
In the figure (a) shows that with increased of chitosan concentrations so the loading capacity and encapsulation efficiency will also increase, while the addition of STPP concentration will decrease of loading capacity and encapsulation efficiency. While in addition of the STPP concentration will decrease loading capacity because the droplets rapidly solidification occurs when the process of ionic gelation \[^{12}\]. In figure (b) shows that increasing of the centrifugation speed will increase loading capacity, because with the increasing of the centrifugation speed, then small-sized particles will also precipitates (not only large-sized particles that precipitates), so the mass of nanoparticles after freeze drying will be more.

Encapsulation efficiency obtained ranged from 99.47%-99.51%, this result is appropriate with Chen et al. (2006)\[^{13}\] who is state that good encapsulation efficiency is at least 80%, because it shows the processes that do not eliminate existing active substances. The high value of the encapsulation efficiency may also be evidenced by the results of morphology in FTIR in figure 2, because andrographolide contained in the sambiloto extract have been encapsulation with chitosan, as evidenced by the existence of andrographolide’s functional group on nanoparticle.

3.6. Controlled drug release of Nanoparticle

The results showed that the cumulative release is 34.55% at the 7th hour. In figure 3 shows that at the 1st to 3rd hour which indicates gastric condition with the pH 1.2 longer than at pH 7.4 which indicates intestinal conditions shows profile release to be faster.

In figure 3 shows that at the 1st to 3rd hour in which indicates gastric condition is slow release. This is because in these conditions, the hydrogen bonds between molecules of Chitosan nanoparticles make getting stronger which causes diffusion into the molecule becomes more difficult, and then followed the release of a drug that lasts longer\[^{14}\]. The addition of surfactants such as tween 80 may increase the endurance of the particles on the acid condition in gastric.

Then on the intestinal conditions pH 7.4 shows the release profiles tend to be faster. That is called burst release i.e. mechanism of drug released actively, indicating the drug is at the surface of the nanoparticles so that chitosan having deprotonation on the surface when mixed with synthetic fluid medium\[^{15}\].

![Figure 3. Release profile of nanoparticle in series](image)

At the Simulated Intestinal Fluid (SIF), the concentration of STPP contributed to the polymer chain conformation more because deprotonation, so chitosan nanoparticle easy damaged \[^{16}\]. Has been previously reported also that on media with pH above 6 ionization of amine reduced drastically \[^{17}\]. This triggered the deprotonation of the amine chitosan and chitosan particles causing crosslink with STPP became unstable and start to degradation as reported on a similar research\[^{18}\].
4. Conclusion
Based on the results of the research, it can be concluded that the results of measurements of the particles distribution have an average of 68.3 nm on a variation of the concentration ratio chitosan:STPP 0.2%:0.1% (g/mL) with the centrifugation speed 8,000 rpm. The variation has loading capacity and efficiency encapsulation each 67.20% and 99.48%, and cumulative release 34.55%.

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