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EFFECT OF CHITOSAN CONCENTRATION ON PHYSICAL CHARACTERISTICS OF EXTRACT ETHANOLIC OF BAY LEAF (*Syzygium polyanthum*) NANOPARTICLE PREPARED BY CROSS-LINKING METHODS

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ABSTRACT
Chitosan is a natural cationic polysaccharide that could form bonds with negatively charged polyanions like sodium tripolyphosphate (STPP) as a crosslinker. One of the important factors to develop nanoparticles is the concentration of polymer. The increased polymer concentration will increase the viscosity of the solution formed, and the size of the nanoparticles created will increase. However, if the amount of polymer is too small, the particles formed are smaller, and aggregation could be formed. In this study, the ethanolic extract of bay leaf (*Syzygium polyanthum*) was used as a drug that has a potent anti-dyslipidemia effect by lowering cholesterol and triglyceride levels. Studies on the ethanolic extract of a bay leaf as an anti-dyslipidemia are still limited. The objective of this research was an investigate effect of the chitosan concentration used 0.6 mg/ml (F1); 1mg/ml (F2); and 1.4 mg/ml (F3) on the physical characteristics of ethanolic extract of bay leaf (*Syzygium polyanthum*) nanoparticles (NSPs) prepared by cross-linking methods. The result of particle size evaluation showed that the particle size was 665.1 nm ± 14.71 (F1); 180.1 nm ± 0.5; and 221.35 nm ± 1.91 (F3), while the polydispersity index F1, F2, and F3 were 0.773 ± 0.152; 0.220 ± 0.016; and 0.212 ± 0.024 respectively. The results of this study found F2 was the most optimal chitosan concentration with particle size under 200 nm, and polydispersity index under 0.5 with positive ζ-potential value. In conclusion, chitosan concentration showed has an effect on the physical characteristics of the nanoparticles.

Keywords: Nanoparticle; Chitosan concentration; Cross-linking; Syzygium polyanthum

1. INTRODUCTION

Chitosan is a natural cationic polysaccharide consisting of (1-4)-2-amino-2-deoxy-D-glucopyranosyl units and could be involved in the ionic interaction with anionic substances (De Robertis et al., 2015). Due to its high biodegradability, bioadhesive, bioactivity, biocompatible, and non-toxic polymer in such a way, chitosan has been widely formulated in the development of drug-delivery systems, such as nanoparticles (Jiménez-Gómez & Cecilia, 2020; Rezaei et al., 2024). One of the essential factors in developing nanoparticles using cross-linking methods is polymer concentration. Increasing the polymer concentration further increases the viscosity of the solution formed, and the size of the nanoparticles created will increase. However, if the amount of polymer is too small, the particles formed are smaller, and aggregation could be formed (Antoniou et al., 2015).There are many methods to develop a nanoparticle, the simple and mild technique for chitosan nanoparticle formation is a cross-linked method. This method is processed while an amino group of chitosan is protonated with a polyanion. Sodium tripolyphosphate

(SSTPP) is the most commonly used polyanion because it is nontoxic and can interact with electrostatic forces between $-NH^{3+}$ from chitosan and $-P_3O_{10}^{5-}$, the group will affect the physicochemical characteristics (Kumar et al., 2011).

In this work, the ethanolic extract of bay leaf (*Syzygium polyanthum)* was used as a drug model that has a potent anti-dyslipidemia effect by lowering cholesterol levels (Harismah, 2017). Currently, conventional medicine has developed using nanoparticles. The results of atorvastatin nanoparticles can increase its safety and effectiveness as an anti-dyslipidemia (Nammi et al., 2004). However, limited reports about nanoparticles from natural ingredients for dyslipidemia have been discussed. The study investigated the effect of chitosan concentration on the physical characteristics of ethanolic extract of bay leaf (*Syzigium polyanthum)* nanoparticles (NSPs) prepared by cross-linking methods. Furthermore, this study aims to optimize the sized particles and polydispersity index (PDI), which would be useful for improving nanoparticle application.

2. METHODS

2.1. Materials

Chitosan with medium molecular weight (190-300 kDa) and DD of 88.5% derived from shrimp shells were purchased from CV. Bio Chitosan (Indonesia). The ethanolic extract of bay leaf (*Syzigium polyanthum)* was obtained from PT. Borobudur Extraction Center (Indonesia) and Sodium tripolyphosphate were purchased from Xilong Scientific Co, Ltd. (China). Other reagents were all commercially available and used as received. Deionized water was used in all experiments.

2.2. Phytochemical Screening

Qualitative analysis of alkaloids, saponins, quinones, flavonoids, and tannins according to the method described in Biological and phytochemical screening of Plants (Farnsworth, 1966). Briefly, containing flavonoids, alkaloids, tannins, quinones, and saponins are determined easily and reaction changes are chosen (Farnsworth, 1966, Depkes, 1995a, 1995b).

2.3. Preparation of NSPs

NSPs were prepared through the following methods. Chitosan 0.6 mg/ml (F1), 1 mg/ml (F2), and 1,4 mg/ml (F3) were dissolved in 1%(v/v) glacial acetic acid and the pH was adjusted up to 4.5±0.2 using 5 M NaOH. A 2500 mg/ml solution of ethanolic extract of bay leaf (*Syzigium polyanthum)* was added and the solution was incubated for 30 minutes in a dark room. 1.4 mg/ml aqueous solution of STPP as cross-linker was added drop-wise to the chitosan solution under magnetic stirring at 800 rpm for 90 minutes at room temperature. The ratio of chitosan: STPP (5:1) was used. Then, all samples were centrifugated at 13.000 rpm for 10 minutes. After centrifugation, the supernatant layer was removed carefully. Then, the sediment was redispersed with deionized water. After that, the NSPs suspension was sonicated in a water bath sonicator for 60 minutes in order to separate the nanoparticles from large particles or aggregates.

2.4. Characterization of NSPs

The resulting NSPs were characterized in the form of particle size, polydispersity index (PDI), and ζ-potential value was measured using Zetasizer® Nano ZS (Malvern Instrumentation Co.).

2.5. Statistical Analysis

Data in this study were initially evaluated by analysis of variance (ANOVA) using SPSS Statistic software (version 22., SPSS Inc. Chicago, IL). Significant results were further analyzed using a range of tests ($P<0.05$) to compare the mean values of the data.

3. RESULTS AND DISCUSSION

NSPs were prepared by the cross-linking method, where electrostatic forces occur between $-NH^{3+}$ from chitosan and $-P_3O_{10}^{5-}$ from SSTPP, then ethanolic extract of bay leaf (*Syzigium polyanthum)* encapsulated within the nanoparticles. Some parameters can be varied during the fabrication of the nanoparticle to vary the size, ζ-potential, and PDI.

3.1. Phytochemical Screening

All of the materials in this study such as ethanolic extract of bay leaf (*Syzigium polyanthum)*, chitosan, STPP, and others were purchased. Furthermore, a phytochemical screening test for the extract was carried out. The aim of this test is to identify and ensure the content of the secondary metabolite compounds in the ethanolic bay leaf extract of *Syzygium polyanthum* (Nasyanka et al., 2022). Table 1 shows the results of the phytochemical screening.

Table 1. The results of ethanolic extract of bay leaf (*Syzigium polyanthum*) phytochemical screening.

No	The Secondary Metabolite Compounds	The Result*
1	Alkaloids	+
2	Saponins	+
3	Quinone	+
4	Flavonoids	+
5	Tannins	+

*(+) positive: contain compounds
(-) negative: does not contain compounds

From Table 1, it can be observed that the extract contains alkaloids, saponins, quinone, flavonoids, and tannins. Similar results were reported by (Kusuma et al., 2011, Widjajakusuma et al., 2019, Widyawati et al., 2021).

3.2. Effect of Chitosan Concentration on Physical Characteristics of NSPs

NSPs are formed when the STPP crosslinker is added to the chitosan ethanolic extract of bay leaf (*Syzigium polyanthum)* solution. The nanoparticle is indicated by the formation of white colloids in each formula during the process. After the settling process, colloids can be observed in each formula. Then, the process was continued to evaluate the physical characteristics of NSPs. The evaluation of physical characteristics carried out included particle size, PDI, and ζ-potential evaluation. **Figure 1** shows the effect of concentration of three different chitosan concentrations, 0.6 mg/ml; 1.0 mg/ml; and 1.4 mg/ml at a constant ratio of chitosan: STPP (5:1) on particle size and PDI.

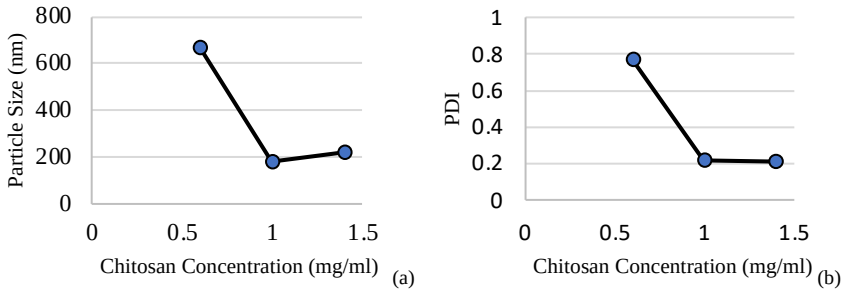


Figure 1. Effect of chitosan concentration on (a) particle size and (b) PDI of NSPs.

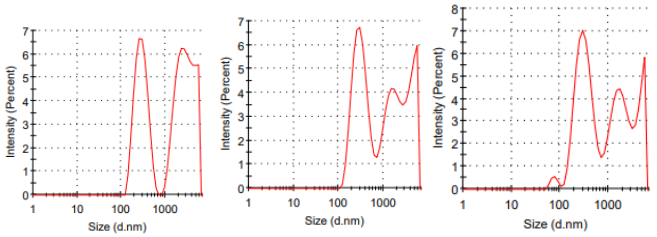
From **Figure 1a** it shows the size particles on F1 (665.10 nm ± 14.71), F2 (180.10 nm ± 0.50), and F2 (221.35 nm ± 1.91) already have a size < 1000 nm. For chitosan concentration in the three formulas, F1 (0.6 mg/ml); F2 (1.0 mg/ml); and F3 (1.4 mg/ml) were different, where especially F2 (180.1 nm ± 0.5) smaller and significantly than F1 (665.1 nm ± 14.71) and F3 (221.35 nm ± 1.91) ($P<0.05$). The PDI of the particles had a similar trend to the particle size

(**Figure 1b**), while the PDI F1, F2, and F3 were 0.773 ± 0.152; 0.220 ± 0.016; and 0.212 ± 0.024 respectively. The optimum condition of the nanoparticle is important to prevent flocculation, since the synthesis of chitosan nanoparticles is affected by various factors, optimizing the preparation condition could be of great importance. Hence, this evaluation shows that the optimum chitosan concentration was found to be 1.0 mg/ml while particle size under 200 nm with a positive ζ-potential value (21.8±1.74). This is similar to other research by (Vaezifar et al., 2013).

3.3. Size Distribution of NSPs

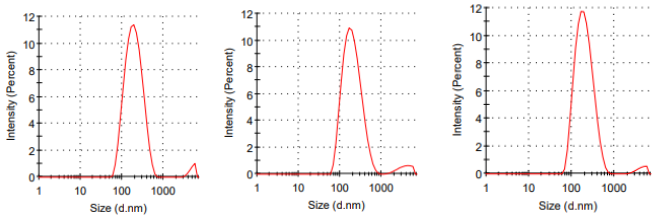
The chitosan concentration has a significant effect on particle size and we observed the sized distribution of three different formulas had different intensities appeared (**Figure 2**). **Figure 2a** shows that F1 has two peaks, which means the NSPs do not distribute well with lower intensity in this chitosan concentration. The different results are shown at F2 and F3 (**Figure 2a** and **Figure 2c**), in which these two groups had an average size ≤200 nm and one peak appeared. These results mean that the two formulas distributed well with higher intensity and proved that different chitosan concentrations could affect the intensity of the size distribution of the nanoparticles. The optimum concentration of chitosan used, the better the peak intensity of the particle size distribution will be (Antoniou et al., 2015).

Formula	Particle Size (nm)	Average Size ± SD
F1	675.5 597.0 654.7	665.1 nm ± 14.71



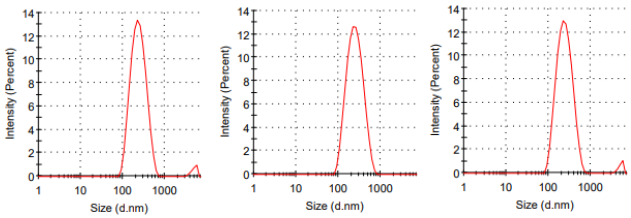
(a)

Formula	Particle Size (nm)	Average Size ± SD
F2	179.6 180.5 180.2	180.1 nm ± 0.5



(b)

Formula	Particle Size (nm)	Average Size ± SD
F3	220 222.7 225.8	221.35 nm ± 1.91



(c)

Figure 2. Size distribution by intensity for particles at different chitosan concentrations (a) F1 (0.6 mg/ml); (b) F2 (1.0 mg/ml), and (c) F3 (1.4 mg/ml) with triple-result respectively.

4. CONCLUSION

In this study, the condition controlling chitosan concentration in NSPs is detailed. The size, dispersion (PDI), and ζ-potential of NSPs are affected by chitosan concentration. Controlling these parameters results is an opportunity for optimizing the nanoparticle for the intended drug delivery system applications. Our results suggested that chitosan concentration affected the physical characteristics of the NSPs and F2 (1.0 mg/ml) was the most optimal chitosan

concentration with particle size under 200 nm, and polydispersity index under 0.5 with a positive ζ-potential value.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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