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Suspension Stability and Characterization of Chitosan Nanoparticle–Coated Ketoprofen Based on Surfactants Oleic Acid and Poloxamer 188

Nur Qadri Rasyid[1], Purwantiningsih Sugita[1*], Laksmi Ambarsari[2], and Gustini Syahbirin[1]

1. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Bogor 16680, Indonesia
2. Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Bogor 16680, Indonesia

[*]*E-mail: purwantiningsih@ipb.ac.id*

Abstract

In this research, ketoprofen was used as a drug model in the preparation of chitosan nanoparticles as a potential drug delivery system through the ionic gelation process with tripolyphosphate (TPP). The particle size analysis (PSA) revealed that the average particle size, polydispersity index (PI), and entrapment efficiency of chitosan nanoparticles prepared with oleic acid were 253.7 nm and 0.375 with drug entrapment efficiency of 73.30%. Those prepared with poloxamer 188 were 242.94 nm and 0.302 with drug entrapment efficiency of 87.89%. Scanning electron microscopy (SEM) analysis showed that the shapes of the nanoparticles, both prepared with oleic acid and poloxamer 188, were intact and spherical. Fourier transform infrared spectroscopy (FTIR) indicated several differences between the spectra of chitosan- and ketoprofen-loaded chitosan nanoparticles; for example, a new peak at the wavenumber 1409/cm indicated the presence of electrostatic interaction between the carboxyl group of ketoprofen and the amino group of chitosan. The chitosan nanoparticle suspension prepared with poloxamer 188 showed smaller increases in turbidity and viscosity than that prepared with oleic acid after 34 d of storage.

Abstrak

Stabilitas Suspensi dan Karakterisasi Ketoprofen Tersalut Nanopartikel Kitosan Berdasarkan Jenis Surfaktan Asam Oleat dan Poloxamer 188. Pada penelitian ini, ketoprofen digunakan sebagai model obat dalam pembuatan nanopartikel kitosan melalui proses gelasi ionik dengan tripolifosfat (TPP). Hasil *Particle Size Analysis* (PSA) menunjukkan ukuran partikel rata-rata dan indeks polidispersitas nanopartikel kitosan dengan surfaktan asam oleat masing-masing adalah 253,75 nm dan 0,375 dengan efisiensi penjerapan 73,30% sedangkan dengan surfaktan poloxamer 188 masing-masing adalah 242,94 nm dan 0,302 dengan efisiensi penjerapan 87,89%. Hasil analisis SEM baik dengan surfaktan asam oleat maupun dengan poloxamer 188 menunjukkan bentuk partikel keduanya adalah bentuk bulat utuh sedangkan berdasarkan spektrum FTIR mengindikasikan bahwa terdapat beberapa perbedaan antara spektrum FTIR kitosan dan nanopartikel kitosan terisi ketoprofen, seperti, munculnya puncak serapan baru pada bilangan gelombang 1409/cm yang menunjukkan adanya interaksi elektrostatik antara gugus karboksilat dari ketoprofen dengan gugus amino kitosan. Analisis stabilitas suspensi nanopartikel menunjukkan suspensi nanopartikel kitosan yang dibuat menggunakan surfaktan poloxamer 188 mengalami kenaikan turbiditas lebih kecil dibandingkan dengan yang dibuat menggunakan surfaktan asam oleat setelah 34 hari penyimpanan.

Keywords: chitosan, ketoprofen, nanoparticles, oleic acid, poloxamer 188

1. Introduction

Ketoprofen is in the group of non-steroid anti-inflammatory drugs (NSAIDs) used to treat inflammation, pain, and rheumatoid arthritis [1]. Ketoprofen has a short elimination half-life of about 1–3 h [2] and a low dissolution rate that requires a higher dosage to maintain a therapeutic level in the patient's blood. Use of ketoprofen dosages greater than 300 mg, however, may cause adverse effects in the upper part of the gastrointestinal tract [3]. Therefore, a drug preparation that can improve the dissolution rate and reduce the dosage to minimize adverse effects is needed.

A nanoparticle drug delivery system holds promise for achieving this end, as this kinds of system can are

deliver drugs to the right site, at the right time, and at the correct dose level [4]. The capability to pass barriers in biological systems is the method's main advantage. The active ingredient in a biological medium can be protected from degradation and delivered to the treatment site in a controlled manner [5]. Chitosan is one of the most abundant naturally occurring biopolymers with a cationic polyelectrolyte nature that is also non-toxic, biocompatible, and biodegradable, making it suitable as a nanoparticle drug delivery system [6].

Nanoparticle formation is not only affected by the material composition and the method used in synthesis, but the addition of surfactant can create a greater number of more stable nanometer-sized particles to agglomeration. Therefore, it is desirable to add a surface-active agent (surfactant) to lower the surface energy of the solution. According to Tojo *et al.* [7], the use of a surfactant in the preparation of nanoparticles can have an effect on the size, PI, and structure of the nanoparticles produced. The objective of this research was to prepare tripolyphosphate-modified chitosan nanoparticle-coated ketoprofen by adding different surfactants as the surface-active agents and determine the stability of the nanoparticle suspension thereafter. Surfactant used in this research is natural surfactant oleic acid and non-ionic surfactant poloxamer 188.

2. Experiment

The main materials used in this research were ketoprofen and chitosan, which were purchased from Kalbe Farma and Bratachem Indonesia. The specifications of the chitosan included the degrees of deacetylation, water, and ash contents, which were 77.26, 9.94, and 0.61%, respectively.

Preparation and Characterization of Chitosan Nanoparticle-Coated Ketoprofen. Ketoprofen-loaded chitosan nanoparticles were prepared by mixing 0.84 mg/mL sodium triphosphate (STPP), chitosan (2.5%) (w/v), 0.2 mg/mL ketoprofen, and 0.8 mg/mL surfactant. Chitosan (50 mL) was added to 20 mL of STPP, while homogenizing at 13,500 rpm for 10 min. Then, 20 mL of ketoprofen was added to the mixture, followed by 20 mL of surfactant, while stirring with a magnetic stirrer for 30 min at 400 rpm. Ultrasonication (20 kHz) was performed on every 25 mL of mixture for 60 min at 20% amplitude. After the ultrasonication, each mixture was centrifuged at 19,900 rpm at 4 °C, for 2 h [8]. Turbidity and viscosity were measured before and after ultrasonication and after centrifugation. The resulting supernatant, which was a suspension of nanoparticles, was separated from the precipitate, and then the particle size and suspension stability were measured after 34 d of storage under conditions room temperature and 4 °C. The chitosan nanoparticle suspension was then spray dried to powder. The characterization of its morphology was conducted with SEM, and the functional groups were analyzed with FTIR.

Efficiency of ketoprofen entrapment in chitosan nanoparticles. Chitosan nanoparticles (25 mg) were dissolved in 50 mL of phosphate buffer (pH 7.2), and then stirred for 24 h and filtered. The absorbance of the resulting filtrate was measured with UV spectrophotometer at λ_{max} 259.8 nm [9]. The absorbance from the measurement was used to determine the ketoprofen concentration on a standard curve. The entrapment efficiency was measured with the following equation (1).

$$EE = \frac{\text{x mg/L} \times \text{1L/1000 mL} \times \text{extraction vol.} \times \text{a mg/b mg}}{\text{Initial ketoprofen mass (mg)}} \times 100\% \quad (1)$$

EE: Entrapment efficiency; x: formula concentration; a: total mass of nanoparticles obtained; and b: nanoparticles mass used to determine the efficiency.

3. Results and Discussion

Preparation of Ketoprofen-loaded nanoparticles based on types of surfactants. Chitosan nanoparticle-coated ketoprofen was prepared by the ionic gelation method with STTP as a crosslinking agent through the homogenization process at room temperature. The mechanism of the chitosan nanoparticle formation is an electrostatic interaction between the positively charged amine group of chitosan and the negatively charged phosphate group of STTP [10]. This interaction creates a stable matrix, renders ketoprofen easier to entrap, and releases it back from matrix [9]. The analysis of turbidity and viscosity of the chitosan nanoparticle suspension showed that ketoprofen-loaded chitosan nanoparticle preparation using poloxamer 188 gave turbidity and viscosity values lower than did oleic acid (Table 1). Different turbidity values were also observed in the research conducted by Sugita *et al.* [11] using 70.6 NTU with oleic acid as the surfactant, and work by Lidiniyah [12] using 5.42 NTU with poloxamer 188 as the surfactant. This was likely because of the decrease in the particle size due to the cavitation phenomenon during ultrasonication that may rupture the molecules into smaller sizes and separate the unruptured, larger particles in the ultrasonication process during centrifugation. In the third stage, the surfactant is used to form an organized group of molecules called micelle, the hydrophilic part of the surfactant in aqueous media, and to associate them with the lipophilic portion in the oil media. According to Schramm *et al.* [13], the formation of micelles in solution is generally seen as a compromise between the tendency of the alkyl chains to avoid contact with the water, and the tendency of the polar parts to maintain contact with the aqueous

environment. Therefore, the addition of surfactant in the synthesis of chitosan nanoparticles can stabilize the particle sizes so the process does not undergo agglomeration. Figure 1 presents an illustration of the role of surfactants in the particle size reduction during the homogenization and ultrasonication stage.

Analysis of the particle size within the suspension using PSA after centrifugation showed that the particle size produced with poloxamer 188 was smaller than that produced with oleic acid (Table 2). A similar result was observed with PI, which showed nanoparticle size uniformity. This difference in size was assumed due to the different hydrophile–lipophile balance (HLB) value of surfactant used. HLB value greatly affects the stability of the particles in a liquid medium. The higher the HLB value of the surfactant, the more able to stabilize the particles present in the water medium. value of poloxamer 188, which is 29, while the HLB of oleic acid, which is only 1, so that poloxamer 188, which has a long hydrophobic tail, will give a higher particle stability because it could form a more compact micelle structure compared to oleic acid in the medium of water. This longer hydrophobic tail could reduce the surface tension during the cavitation process and result in more stable nanoparticles. The percentage of entrapped ketoprofen in the chitosan nanoparticle matrices of poloxamer 188 was higher than that of oleic acid (i.e.,87.89% and 73.30% respectively), as entrapment efficiency was correlated with particle size. The smaller the particle size, the larger the surface area, which resulted in an increase of entrapment capability. This result also corresponded to a study by Sugita *et al.* (2010) [14], which demonstrated that the use of Tween 80 (HLB = 15) improved the encapsulation efficiency and produced larger nanometer-sized particles, about 100–1,000 nm larger than what was produced using Span 80 (HLB = 4.3).

Characteristics of morphology and chitosan nanoparticle structure. The SEM analysis of the ketoprofen-loaded chitosan nanoparticles prepared with poloxamer 188 and oleic acid showed intact, spherical particles, indicating that the chitosan nanoparticles were

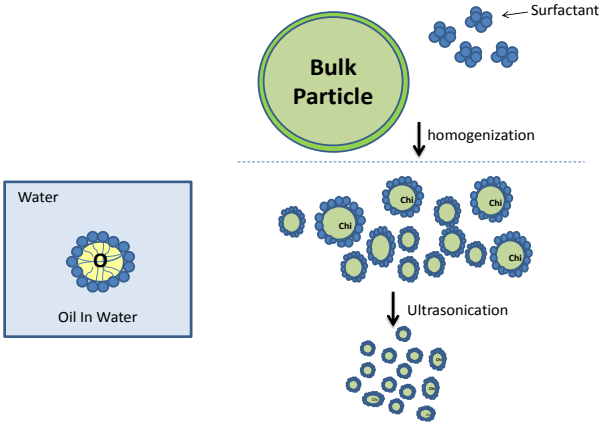

Figure 1. Illustration of the Role of Surfactant in Reducing the Particle Size

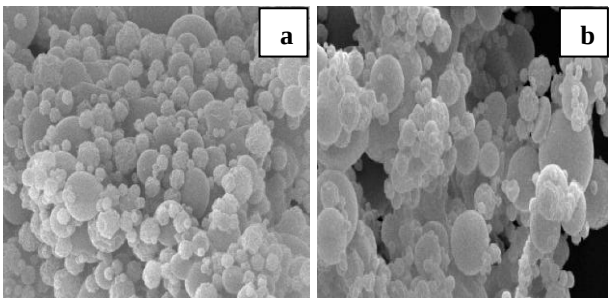

Figure 2. SEM of Ketoprofen–loaded Chitosan Nanoparticle with Surfactant (a) Poloxamer 188 and (b) Oleic Acid at Magnification of 2000X

Table 1. Relation between Types of Surfactant Used and Turbidity and Viscosity

Measurement condition	Turbidity (NTU)		Viscosity (cp)	
	Poloxamer 188	Oleic Acid	Poloxamer 188	Oleic Acid
Before ultrasonication	120	280	146.6	162.5
After ultrasonication	73	173	70.7	94.1
After centrifugation	57	110	56.6	62.7
Percentage reduction (%)	47.50	39.28	38.60	38.58

Table 2. Number of Nanoparticles, Polydispersity Index, and the Entrapment Efficiency of Different Surfactants

Surfactant	Average Particle Diameter (nm)	Range of Particle Diameter (nm)	Σ Nano (%)	EE (%)	IP
Oleic Acid	253.75	35.49–1549.23	93.10	73.30	0.37
Poloxamer 188	242.94	38.91–1230.59	99.50	87.89	0.30

loaded with ketoprofen (Figure 2). According to Sugita *et al.* [14] and Wahyono *et al.* [9], the shape of the unloaded chitosan nanoparticles was wrinkled and slightly flat. However, the ketoprofen-loaded chitosan nanoparticles had an intact spherical shapes. The chitosan nanoparticles prepared with poloxamer 188 showed a smaller and more uniform particle size compared to those prepared with oleic acid. This result was also seen in the size percentages, amounts, and PI of nanoparticles obtained from the PSA analysis.

FTIR analysis was used to characterize the interaction of functional groups in the nanoparticles. FTIR spectra of chitosan, ketoprofen, and ketoprofen-loaded chitosan nanoparticles prepared with oleic acid and poloxamer 188 are shown in Figure 3. In the spectrum of chitosan, there were three specific peaks at wavenumbers 3,432/cm (-OH), 1,074/cm (C-O-C) and 1,651 cm^{-1} (NH_2) [15]. The spectrum of chitosan was different from the spectrum of ketoprofen-loaded chitosan particles. There was a shift in the peak of NH_2 of chitosan from 1,651/cm to 1,640/cm in the ketoprofen-loaded chitosan nanoparticles, which was accompanied by the appearance of a new peak at 1,562/cm as the result of electrostatic interaction between the amine group of chitosan and the phosphate group of TPP. The difference in the spectra of ketoprofen and ketoprofen-loaded chitosan nanoparticles was in the appearance of a new peak at 1,409/cm due to the electrostatic interaction between the carboxylic group of ketoprofen and the amino group of chitosan forming a carboxylate salt [9] in the ketoprofen-loaded chitosan nanoparticles. Additionally, the appearance of a peak at 1,041/cm of the ketoprofen-loaded chitosan nanoparticles revealed the presence of the P-OH group of TPP [16].

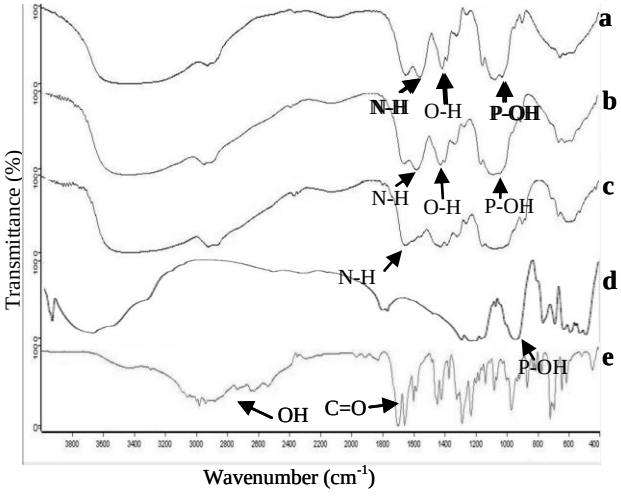

Figure 3. FTIR Spectrum (a) Chitosan Nanoparticles Prepared with Poloxamer 188, (b) Oleic Acid, (c) Spectrum of Chitosan, (d) Spectrum of STPP and (e) Spectrum of Ketoprofen

Chitosan nanoparticle suspension stability. The turbidity and viscosity stability of the ketoprofen-loaded chitosan nanoparticle suspension was based on the types of surfactants used in the preparation, in storage conditions (room temperature and 4 °C) for 34 d. Figure 4 shows that the use of poloxamer 188 as the surfactant could slow the increase in turbidity of the resulting ketoprofen-loaded chitosan nanoparticle suspension compared to oleic acid. The percentage increases in turbidity of chitosan nanoparticle suspension for 34 days with surfactant poloxamer 188 at room temperature and 4 °C respectively were 44.08% and 42.17%, while the oleic acid surfactants were 51.40% and 51.07% respectively. The increased turbidity of the two suspensions were probably due to the agglomeration of particles in suspension.The result of PSA showed that the particle size of the chitosan nanoparticle suspension prepared with oleic acid increased to >300 nm, while that prepared with poloxamer 188 was still in the initial range of nanoparticle size (i.e., 200–300 nm). This may have been because the high value of HLB of poloxamer 188 reduced agglomeration during storage, which could have assisted in stabilizing the particle size. However, the turbidity analyses for both chitosan nanoparticle suspensions with poloxamer 188 and oleic acid surfactants stored at room temperature and 4 °C did not show any significant difference. Therefore, it appears that the suspension could be stored at room temperature or 4 °C. This result was similar to that of Joseph and Sharma [17], where cytarabine-loaded chitosan nanoparticles that maintained stability at room temperature and 4 °C after a 34 d storage.

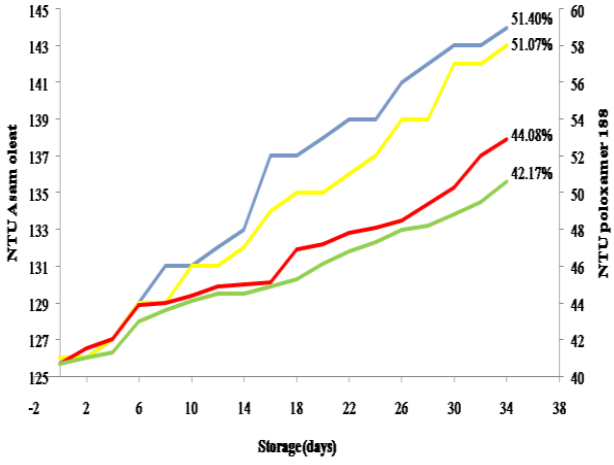

Figure 4. Increased Turbidity of Ketoprofen-loaded Chitosan Nanoparticles with Surfactant (—) Oleic Acid at Room Temperature, (—) Oleic Acid at 4 °C, (—) Poloxamer 188 at Room Temperature and (—) Poloxamer 188 at 4 °C based on Period of Storage

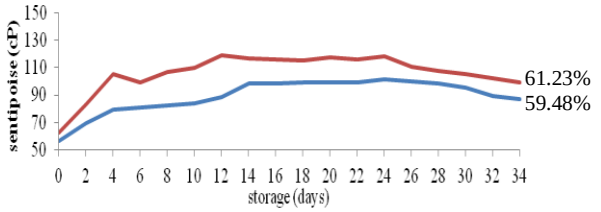

Figure 5. Increased Viscosity of Ketoprofen-loaded Chitosan Nanoparticles with Surfactant ( ) Oleic Acid and ( ) Poloxamer 188 based on Storage Period

The viscosity of the stability of chitosan nanoparticles with poloxamer 188 or with oleic acid showed that the suspension viscosity of chitosan nanoparticles with surfactant poloxamer 188 increased to 59.48% during storage, while the oleic acid viscosity was 61.23% (Figure 5). This was caused by particle agglomeration due to surfactant activity decrement. This result supports the hypothesis of Duan *et al*. [18]. However, in this study, the viscosity of poloxamer 188 on day 26 and oleic acid on day 22 decreased with the hydrolysis of chitosan. According to El-Hefian *et al.* [19], chitosan which is stored for a long time in organic acid solution, will cause viscosity decrement due to the hydrolysis of chitosan.

4. Conclusions

Chitosan nanoparticles prepared with poloxamer 188 were smaller and more numerousa, thereby resulting in a higher ketoprofen entrapment efficiency of chitosan nanoparticles than oleic acid. The addition of poloxamer 188 in the preparation of ketoprofen-loaded chitosan nanoparticles resulted in higher stability than oleic acid during 34 d of storage. The storage condition based on increased turbidity showed that the chitosan nanoparticle suspension could be stored at room temperature and 4 °C. SEM analysis showed that the nanoparticles obtained with poloxamer 188 and oleic acid were intact spherical nanoparticles. FTIR analysis showed that there were some differences in the spectra of chitosan, ketoprofen, and ketoprofen-loaded nanoparticles.

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