



RESEARCH ARTICLE

PREPARATION AND CHARACTERIZATION OF THYMOQUINONE NANOPARTICLES PEGYLATED AS DRUG DELIVERY SYSTEM

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Abstract

Objective: Thymoquinone is a main component of Black Cumin (*Nigella sativa* Linn.) with various pharmacological activities, but has poor stability and bioavailability. The purpose of this study was to carry out the preparation and characterization of thymoquinone nanoparticles PEGylation.

Methods: The Thymoquinone nanoparticles (TQ-NP) were made with PEGylation using PEG 6000 with the concentrations on each preparation of 3 mM (A), 4 mM (B), and 5 mM (C) then were evaluated by the parameter of yield percentage Entrapment Efficiency (EE) and Drug Loading (DL), drug release, size and distribution particle, morphological analysis and Fourier Transform-Infrared spectrophotometer (FTIR).

Results: Thymoquinone nanoparticle was PEGylated with PEG 6000 has the highest efficiency entrapment of 99.9718±0.029% in formula A, with the capacity of drug loading 0.66%. Formulation A release 99.9718±0.029% of Thymoquinone at 50 minutes. The morphological observations with Scanning Electron Microscope (SEM) showed spherical nanoparticles morphology.

Keywords: Black Cumin, Nanoparticles, PEG 6000, Pegylation, Thymoquinone.

INTRODUCTION

Black cumin mostly used as a food additive and has properties to treat several diseases due to its various chemical properties¹. Black cumin oil has various therapeutic benefits such as dysentery, headaches¹, gastrointestinal problems^{2,3}, eczema, hypertension⁴, and obesity, etc. Thymoquinone (TQ) is one of the major active chemical component of black cumin oil (*Nigella sativa* Linn)⁵. TQ can increase the immune system of patients with bronchial asthma due to allergies, and the main properties as an anti-inflammatory^{1,6} and antioxidants. In a study conducted by Salim *et al.*, showed that TQ has an IC value of 1.5 ±0.04 µg/mL⁷. Thymoquinone as an anti-inflammatory works by inhibiting the cyclooxygenase (COX) and lipoxygenase (LO) pathways⁶. In addition, black cumin oil also contains chemical components such as, nigellienine, nigellamine-n-oxide, essential oils, fatty oils, alkaloid group compounds, saponins, steroids, isokuinolin alkaloids, oleats, and linolenate⁸. Thymoquinone is an essential oil that has volatile properties, low melting point and is easily oxidized, so it will be difficult to formulate into a pharmaceutical

dosage form⁹. In addition, thymoquinone is also difficult to dissolve in water, and has a poor bioavailability¹⁰. To overcome this problem a nanoparticle preparation was developed as a thymoquinone carrier¹¹. Nanoparticles are colloidal particles that range in size from 1-100 nm. The drug is dissolved, adsorbed, encapsulated or attached to the nanoparticle matrix¹². The advantage of nanoparticles is that it increases the bioavailability of the drug, small doses, reduces side effects, increases the surface area to produce rapid solubility of active ingredients¹³. If the dissolution rate is high, the absorption and bioavailability of the drug will also be better¹⁴. Within a few years, many methods have been designed in the development of formulations to improve the characteristic and pharmacokinetics properties of a drug compound to produce the maximum benefit of therapeutic action¹². One of them is PEGylated nanoparticles.

Pegylation is a modification of protein, peptide and non peptide molecules by forming bonds or links with one or more chains of polyethylene glycol (PEG). Polyethylene glycol (PEG) is a polymer that has been approved by the FDA in the application of

nanomedicine and biomedicine. A good nanoparticle formulation should have a high drug loading capacity¹⁵. The molecular weight of polyethylene glycol (PEG) used can affect the loading capacity of the drug so that it is necessary to optimize the type and concentration of

PEG used based on its molecular weight¹⁶. Based on this, it is necessary to do research on the preparation and characterization of thymoquinone nanoparticles so that they can be used for drug delivery.

Table 1: Thymoquinone nanoparticles optimization.

Materials	Concentrations			Formed Solid Particles
	P A	P B	P C	
Thymoquinone	1 mg	1 mg	1 mg	-
PEG 400	72 mg	-	-	No
PEG 4000	-	72 mg	-	No
PEG 6000	-	-	72 mg	Yes
Tween 80	0.03 ml	0.03 ml	0.03 ml	-
Ethanol	Ad 5 ml	Ad 5 ml	Ad 5 ml	-

SUBJECTS AND METHODS

Thymoquinone (TQ) was obtained from Sigma Aldrich USA. The TQ was prepared TQ was prepare into the form nanoparticles PEGylated. In this study, the TQ was quantified using spectrophotometer UV-Vis.

Standard Solution preparation

A stock solution of 100 ppm of Thymoquinone concentration was made by weighing 10 mg of TQ and dissolving it in 100 mL of phosphate buffer pH 7.4. The solution is used to determine the maximum wavelength of TQ. The λ_{max} was observed with a spectrophotometer UV-VIS at 200-400 nm.

Preparation of Thymoquinone Nanoparticle (TQ-NP)

Nanoparticles are made by the pegylation method. A series of concentrations of PEG 400, PEG 4000 and PEG 6000 were made as polymers, Tween 80 as a surfactant in the optimization of nanoparticles can be seen in Table 1. Each formula was made as much as 5 mL with 1 mg of TQ. Dissolved 1 mg of TQ in 70% ethanol. Tween 80 added 0.03 mL into the mixture until it was homogeneous, then the mixture was slowly dripped into polyethylene glycol (PEG) while being stirrer at a speed of 700 rpm for 6 hours. The TQ-PEG dispersion was then evaporated to remove solvents and made into powder with freeze drying technique to obtain TQ-NP PEGylated⁵.

Characterization of TQ-NP

TQ-NP Morphology

Morphological observations of TQ-NP were carried out using Scanning Electron Microscope (SEM). The TQ-NP were dropped over cooper grid then coated with an auto carbon coated (JOEL JEM, Japan) tool for 5 seconds, dried at room temperature for 24 hours. TQ-NP was analyzed by accelerating the voltage at 120 kV and magnification of 60,000.

Particle Size

TQ-NP particle size was determined using a NicompTM 380 ZLS Submicron particle size analyzer. A total of 2 drops of pH 4.0 TQ-NP added 5.0 mL aquadestillata, then mixed by flipping through a conical tube. After that, 3.0 mL was taken and put into cuvettes to analyze distribution and particle size.

The Entrapment Efficiency (EE) and The Loading Drug

Total 5 mg of TQ-NP were centrifuged at room temperature with 15.000 rpm a, for 30 minutes and then absorbance was measured with UV-Vis spectrophotometry. Entrapment Efficiency of TQ-NP was calculated by the equation:

$$\%EE = \frac{\text{drug Total} - \text{Free drug}}{\text{Drug Total}} \times 100$$

Drug Release Study

The release of TQ-NP carried out *in vitro* using Franz diffusion cells, the membrane used was removable python morolus skin. The medium fluid in the receptor compartment used was a phosphate buffer pH of 7.4 and maintained at a temperature of $35 \pm 0.5^\circ\text{C}$ as much as 50 mL. The snake skin is then placed between donor compartments with receptor compartments, samples weighed as much as 5 mg are applied to the surface of the skin. Medium liquid is flowed through the bottom of the skin membrane with the help of a magnetic stirrer at a speed of 100 rpm. Sampling was carried out at the 10, 20, 30, 40, 50, 60, 70, 80, 90, 110, and 120 minute where 5 mL samples were taken from the receptor compartment using a syringe and immediately replaced with a 5 mL medium solution. The samples were then measured for absorbance using UV-Vis spectrophotometry at a maximum wavelength of 260 nm.

RESULTS AND DISCUSSION

The development of nanoparticles using thymoquinone as an active ingredient is based on volatility, low melting point and easily oxidized¹⁰. Thymoquinone is also difficult to dissolve in water, so it has a small bioavailability⁹. Therefore, thymoquinone is made in the form of nanoparticles to improve the bioavailability of drugs, improve the physical properties of chemicals¹⁰ and protect medicinal ingredients so as to provide an effective therapeutic effect¹⁷. In this study, the resulting nanoparticle TQ-NP was characterized by evaluating nanoparticle morphology, size and distribution of particles, measurement of entrapment efficiency (EE) and drug loading (DL), and nanoparticle release. In this study thymoquinone was prepared to form nanoparticles with PEGylated method using PEG 6000 as a polymer. PEG was chosen as a polymer used because it was 'safe' in the body and

approved for use as excipients in many pharmaceutical formulations¹⁶. PEG has been widely used in various nanoparticle systems to increase surface hydrophilicity and half-life by interacting with blood and mononuclear phagocyte cell systems. Pegylation is formed because physical adsorption or covalent grafting results in a layer of PEG on the surface of the particles so that it can increase the stability of the medicinal material¹⁷.

Table 2: Entrapment efficiency (EE) of TQ-NP.

Preparations (TQ:PEG 6000)	% EE
A (1:3)	99.9718±0.029
B (1:3)	99.9628±0.026
C (1:3)	99.9363±0.049

To improve the properties of a drug that has poor bioavailability and its solubility can be made into a form of nanoparticles. Some studies have shown both the shape of the PEG configuration and its molecular weight affects the pharmacokinetic properties and biological activity of the drug. It is very important to choose PEG with different molecular weights¹⁸. In this study used PEG with different molecular weights are PEG 400, PEG 4000, and PEG 6000 and loading with 3 mM of TQ. The Optimization was done by making preparations with a comparison of PEG polymers with different molecular weights, the type of PEG used (400, 4000, 6000) with a concentration of 3 mM. In making the pegylation method thymoquinone and PEG were dissolved in 70% ethanol, then tween 80 was added as a surfactant as much as 0.03 mL, the distrer was at 700 rpm, dropped thymoquinone and distrerer dispersion for 6 hours, using a rotary evaporator at

40°C at a speed 50 rpm and mixed with freeze drying technique to form thymoquinone nanoparticles.

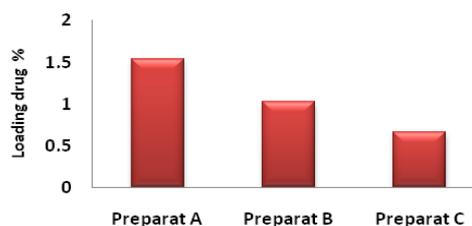


Figure 1: Percentage yields of TQ-NP.



Figure 2: Drug Loading (DL) Histogram of Thymoquinone nanoparticles.

The optimization parameter is to see the formation of particles after being pollinated by the technique of freeze drying.

The Efficiency of Entrapment (EE) and the Loading of Drug (LD)

The Efficiency of Entrapment (EE) shows the ability of polymers to entrapment drugs, while DL shows the ability of drugs to be absorbed into the polymer matrix. EE can be influenced by the concentration of the polymer used in making nanoparticles¹⁹. The EE test results show that all preparations have % EE, which are 99.9718±0.029%, 99.9628±0.026%, and 99.9363±0.049%, respectively.

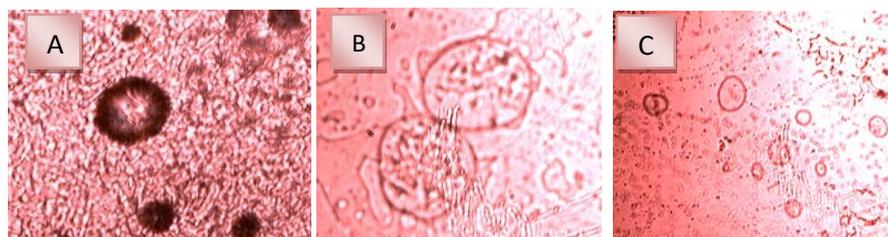


Figure 3: The micrograph of TQ-NP: A (3 mM), B (4 mM), C (5 mM) magnification 40 using an irmeco® microscope.

From the results above it can be seen that the higher the polymer concentration will cause the EE value to be low. This can be caused by the high concentration of polymers that will produce a solution with high viscosity so that the drug ingredients will be difficult to diffuse into the polymer matrix¹⁸. Research conducted by Odeh *et al.*,²⁰ who conducted a study of the efficiency of loading timoquinone in liposomes showed a 90% absorption efficiency of timoquinone and the use of Triton X-100 showed that only 49.6% of the drug was absorbed, the value was smaller than the results of research that has been done.

The results of the DL test showed that the preparations A (3 mM), B (4 mM), and (5 mM) were 1.54%, 1.02%, and 0.66% respectively. From these results it is known that preparation A has a high DL value compared to

preparations B and C. An ideal nanoparticulate system has a high drug loading capacity thereby reducing the material used for drug delivery¹⁸. Drug and drug loading is very dependent on drug solubility in materials or polymers²², polymer composition, polymer molecular weight, and drug and polymer interactions²³.

Particles Size

Particle size is very important characteristics in nanoparticle systems. The particle size will determine the drug distribution *in vivo*, the fate of the drug in the biological system and its toxicity and the ability to target drugs in the nanoparticle system²⁴. The size and distribution of particle also affects drug absorption, drug release and stability of the nanoparticles¹⁶. It has been found that particle size affects drug release. Small particles produce a larger surface area. As a result,

most drugs that are inserted will spread to the surface of the particles to facilitate drug release faster. Instead, the drug will experience slower diffusion in larger²⁴. Measurement of size and distribution of nanoparticles using preparations that have good percent EE and *in*

vitro release values. The results showed that preparation A had an average particle size of 10 nm, which is in the range of size of the nanoparticles (10-100 nm).

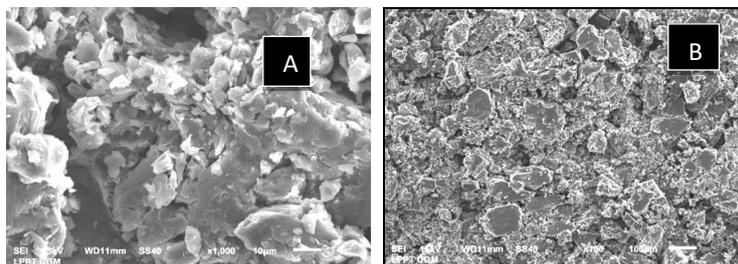


Figure 4: Morphology of thymoquinone nanoparticles using Scanning Electron Microscopy (SEM) with A (1000x magnification) and B (100x magnification).

The Polydispersity Index is a parameter to determine the particle size distribution of nanoparticles with a range that can be absorbed by the PEG 6000 polymer matrix. Preparations A (3 mM) have IP> 0.5 which is 0.350. According to Avadi in 2010, particles with IP> 0.7 have a very wide size distribution. The smaller IP, shows the particle size is uniform. So that it can be concluded that thymoquinone nanoparticles formed monodispersion or relatively homogeneous.

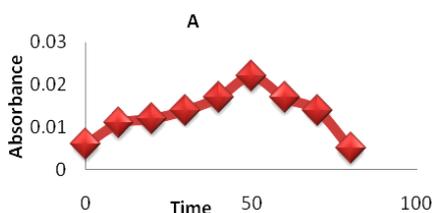


Figure 5: *In-vitro* release curve for preparation A with a concentration of 3 mM.

TQ-NP Morphology

The morphology of Thymoquinone nanoparticles aims to see the shape and morphology of the nanoparticles formed using the irmeco® microscope and Scanning Electron Microscopy (SEM). Based on the results of observations using the irmeco® microscope shown in Figure 1, the thymoquinone nanoparticles formed have a round shape. Scanning Electron Microscope (SEM) is a type of electron microscope that provides a picture of the surface of a sample by scanning using a high-energy electron beam. Electrons interact with sample-forming atoms that produce signals containing information about the surface topography of the sample. The results of observations on preparations A with a concentration of 3 mM showed a spherical shape on the surface of the particles.

TQ-NP *in vitro* study

To develop a nanoparticulate system, biodegradation of polymers and the ability of drugs to release from the system are important considerations¹⁶. In general, the level of drug release depends on: 1. drug solubility¹⁵; 2. surface desorption from adsorbed/ bound drugs; 3. drug diffusion through the nanoparticle matrix²⁴. The erosion /degradation of the nanoparticle matrix and 5. the combination of the erosion/diffusion process²¹.

Solubility, diffusion and biodegradation of matrix materials regulate the release process¹⁸.

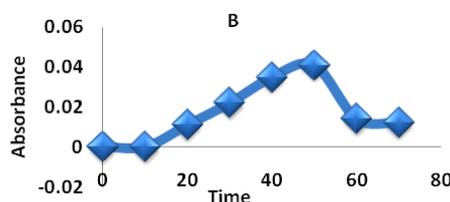


Figure 6: *In-vitro* release curve of the 4 mM B preparations.

The nanoparticle diffusion release test was carried out using a Franz diffusion cell with phosphate buffer pH 7.4 solution. The medium given describes the system of blood flow under the skin. From the results of Thymoquinone release from nanoparticles diffusion can be seen in the figure.

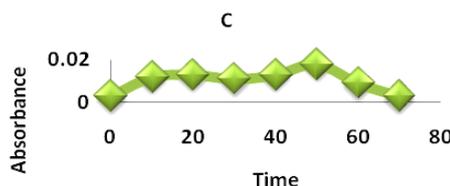


Figure 7: *In-vitro* release curve for C preparations with a concentration of 5 mM.

The release of the drug can be caused by the presence of drugs that are on the surface of the nanoparticles. The results of the studies in Figure 7, Figure 8, and Figure 9 showed that the highest drug release occurred after 50 minute and no release at 90 minutes, from the release of preparation A had a longer release time than preparations B and C (Figure 4, and Figure 5).

In-vitro release graphics for C preparations with a concentration of 5 Mm. The results showed that in Figure 4 and Figure 5, the highest drug release occurred in the 50th minute and no release occurred in the 70th minute.

CONCLUSIONS

Thymoquinone has been prepared into nanoparticles PEGylated using polyethylene glycols 6000 at a

concentration of 3 mM, 4 mM, and 5 mM. Based on the characterization of the entrapment efficiency of nanoparticles obtained the highest adsorption efficiency contained in preparations A 99.9718 ± 0.029 , and preparations B and C respectively 99.9628 ± 0.026 , and 99.9363 ± 0.049 .

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AUTHOR'S CONTRIBUTION

Hasrawati A: writing original draft, literature survey. **Rizaldi I:** methodology, formal analysis. **Bakri NA:** formal analysis, conceptualization. **Febrianti D:** data curation, investigation. **Mursyid AM:** methodology, conceptualization. All authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

No conflict of interest, associated with this work.

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