Original Research Article

PREPARATION AND CHARACTERIZATION OF THYMOQUINONE NANOPARTICLES PEGYLATED AS DRUG DELIVERY SYSTEM

ABSTRACT

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

Methodes: The Thymoquinone nanoparticles 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: Preparation A has the highest efficiency entrapment of 99.9718 ± 0.029%, with the capacity of drug loading 0,66%, and the best drug release. The morphological observations with Scanning Electron Microscope (SEM) showed spherical nanoparticles morphology. The analysis of FT-IR showed that the Thymoquinone nanoparticles had similar characteristics with its coating polymer.

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

INTRODUCTION

Thymoquinone (TQ) is a bioactive compound derived from black seed oil (Nigella sativa Linn.) which grows in the Mediterranean region and in Western Asian countries. Black cumin oil has various therapeutic benefits such as dysentery, headaches, gastrointestinal problems, eczema, hypertension, and obesity, etc[1,2,3].Thymoquinone can increase the immune system of patients with bronchial asthma due to allergies, in addition to its main properties as an allergy and anti-inflammatory[2,3,4,5,6].

The chemical content of black cumin (*Nigella sativa* Linn.) Is thymoquinone, thymohidroquinone, nigellienine, nigellamine-n-oxide, essential oils, fatty oils, alkaloid compounds, saponins, steroids, isoquinolin, oleic, and linolenic alkaloids. The main content of black cumin (Nigella sativa Linn.) Is Thymoquinone (TQ), Dithymoquinone (DTQ), and Thymol (THY) which acts as an antioxidant[2,3].

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 form[7]. In addition, thymoquinone is also difficult to dissolve in water, and has a poor bioavailability[7,8]. To overcome this problem a nanoparticle preparation was developed as a thymoquinone carrier[9,]

Nanoparticles are colloidal particles that range in size from 1-100 nm. The drug is dissolved, absorbed, encapsulated or attached to the nanoparticle matrix. 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 ingredients in the fluid environment, such as in the digestive tract. Dissolution rates along with better absorption and bioavailability[9,10,11]. Within a few years, many methods have been designed in the development of formulations to

improve the pharmaseutic properties and pharmacokinetics of a drug compound to produce the maximum benefit of therapeutic action. 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 capacity. 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 weight[10,11,12,13,14]. 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.

SUBJECTS AND METHODS

Thymoquinone was obtained from Sigma Aldrich USA.The TQ was preparedTQ was prepare into the form oof d nanoparticles PeGylatedusing PEG. Inthis study, the TQ was quantified using spechtrofotometer UV-Vis.

Standard Solution preparation

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

Preparationof *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 thymoquinone. Dissolved 1 mg of thymoquinone 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 sterilized at a speed of 700 rpm for 6 hours. The TQ-PEG dispersion was then rotated to remove solvents and made into powder with freeze drying technique to obtain classified TQ-NP.

Characterization of TQ-NP

a. TQ-NP Morphology

Morphological observations of TQ-NP were carried out using Scanning Electron Microscope (SEM). The TQ-NP were dropped over cooper gird 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.

b. Particle Size

TQ-NP particle size was determined using a NicompTM 380 ZLS Submicron particlesize analyzer. A total of 2 drops of pH 4.0 TQ-NP added 5.0 mL aquadest, 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.

c. The Entrapment Efficiency (EE)and Drug Loading

5 mg of TQ-NP were centrifuged at 15,000 rpm, for 30 minutes and then absorbance was measured with UV-Vis spectrophotometry. Entrapment Efficiency of TQ-NPwas calculated by the equation:

$$\%EE = \frac{(\text{drug Total} - \text{Free drug})}{Drug\ Total}\ x\ 100$$

d. **Yield Value Percentation**

Weighed Thymoquinone nanoparticles and then determined the weight percentage of the material added, namely the weight of the drug and the polymer used.

$$PY(\%) = \frac{The\ weight\ of\ NP}{Total\ Solid\ Weight\ (PEG + TQ)} x100\%$$

e. **In Vitro Study (Drug Release)**

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 ± 0.5°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.

f. FT-IR Analysis

interactions with polymers were determined by IR spectroscopy. 1.0-2.0 grams of thymoquinone, PEG 6000 and TQ-NPwas mixed with potassium bromide. Small amounts of the powder are compressed into thin semitransparent granules using pressure. IR spectra are scanned from 750-4000 cm-1.

RESULTS AND DISCUSSION

The development of nanoparticles using thymoquinone as an active ingredient is based on volatility, low melting point and easily oxidized, thymoquinone is also difficult to dissolve in water, so it has a small bioavailability[7,8,9] Therefore, thymoquinone is made in the form of nanoparticles to improve the bioavailability of drugs, improve the physical properties of chemicals and protect medicinal ingredients so as to provide an effective therapeutic effect[12,13,14].

Table1. Thymoquinone nanoparticles optimization

No	Materials	Concentrations			Formed Solid Particles
		P A	P B	P C	
1	*Thymoquinone*	1 mg	1 mg	1 mg	-
2	PEG 400	72 mg	-	-	No
3	PEG 4000	-	72 mg	-	No
4	PEG 6000	-	-	72 mg	Yes
5	Tween 80	0.03 mL	0.03 mL	0.03 mL	-
6	Etanol	Ad 5 mL	Ad 5 mL	Ad 5 mL	-

In this study, the resulting nanoparticleTQ-NP was characterized by evaluating nanoparticle morphology, size and distribution of particles, measurement of entrapment efficiency (EE) and drug loading (DL), yield value percentage , nanoparticle release, and Fourier Transform-Infrared Spectroscopy analysis (FT- IR).

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

No	Preparations (TQ:PEG 6000)	% EE
1	A (1:3)	99.9718 ± 0.029
2	B (1:3)	99.9628 ± 0.026
3	C (1:3)	99.9363 ± 0.049

In this study thymoquinone was prepared to form nanoparticles using the pegylated method using polyethylene glycol (PEG) as a polymer. The technique developed is expected to increase drug delivery and lack of drugs. PEG was chosen as a polymer used because it was 'safe' in the body and approved for use as excipients in many pharmaceutical formulations. 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 material[16,17,18]

One way to improve the properties of drugs that have poor bioavailability and solubility is formed thymoquinone nanoparticles. Several studies have shown that molecular weight and PEG configuration not only affect pharmacokinetic properties but also biological activity, so it is very important to choose PEG with different molecular weights. PEG is a constituent of nanoparticles so it is optimized between molecular weight (400, 4000, and 6000) and concentration of 3 mM.

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 0C at a speed 50 rpm and mixed with freeze drying technique to form thymoquione nanoparticles. The optimization parameter is to see the formation of particles after being pollinated by the technique of freeze drying. The optimization results showed that only PEG 6000 produced powder (solid particles), can be seen in table 1.

Yield Percentage

Determination of the percentage of yield shows the efficiency of the method used in combining polymers, cross-linkers and medicinal ingredients by looking at the amount of nanoparticle powder produced. The yield percentage is obtained from the comparison between the nanoparticles produced with the amount of the material used in making these nanoparticles. The percentage yield depends on the concentration of the polymer used. High polymer concentrations cause the yield percentage[22,23].

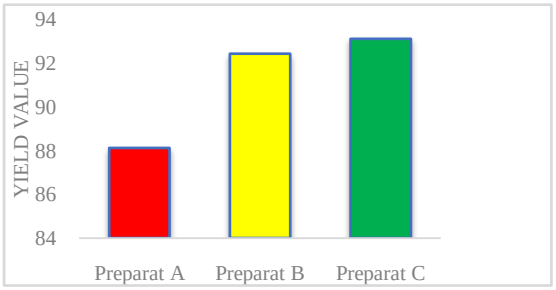


Figure 1. The percentage yields of TQ-NP

From the results of the study, the results obtained in Figure 1 show that in preparation A has a percentage value of yield value of 88.12%, in preparation B 92.43% and in preparation C has the largest percentage yield of 93.11%.

***Entrapment Efficiency* (EE) dan *Drug Loading* (DL)**

Entrapment Efficiency (EE) shows the ability of polymers to absorb 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 nanoparticles. 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. 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 matrix[23,24,25,26].

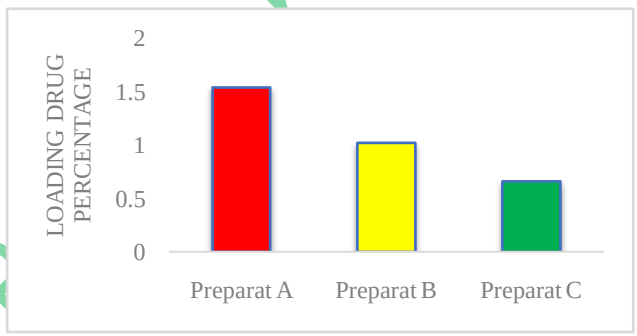


Figure 2. Drug Loading (DL) Histogram of Thymoquinone nanoparticles

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 delivery. Drug and drug loading is very dependent on drug solubility in materials or polymers, polymer composition, polymer molecular weight, and drug and polymer interactions[18,22,23,24].

Particles Size

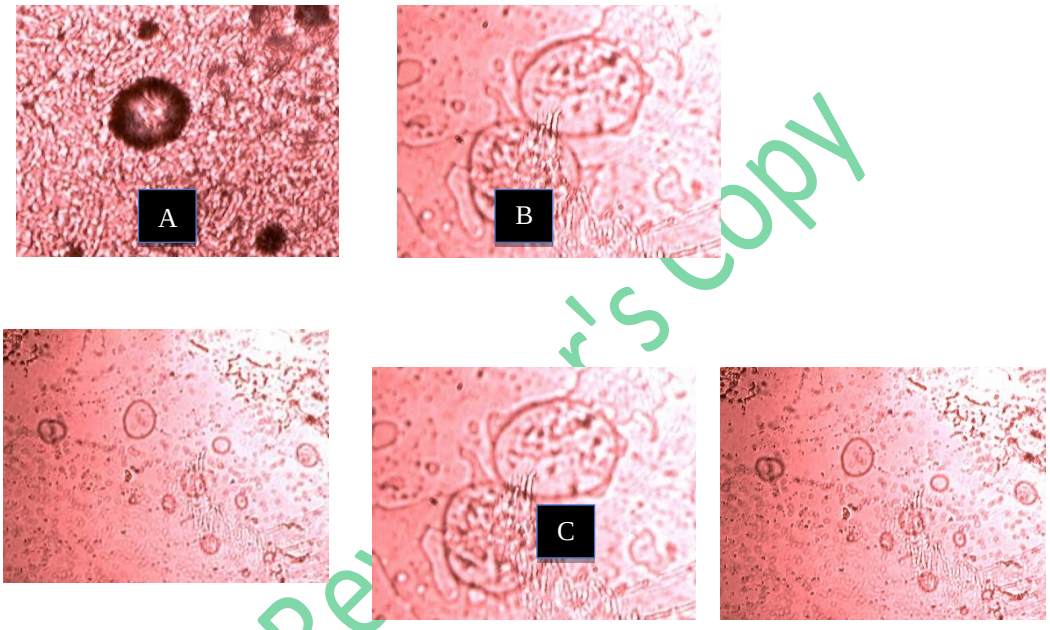
Particle size are very important characteristics in nanoparticle systems. The particle size will determine the drug distribution in vivo, the fate of the drug in the biological system and its toxicity and the ability to target drugs in the nanoparticle system. The size and distribution of particle also affects drug absorption, drug release and stability of the nanoparticles[15].The main application of nanoparticles is drug release and drug targeting. 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 larger [18,19,24,27].

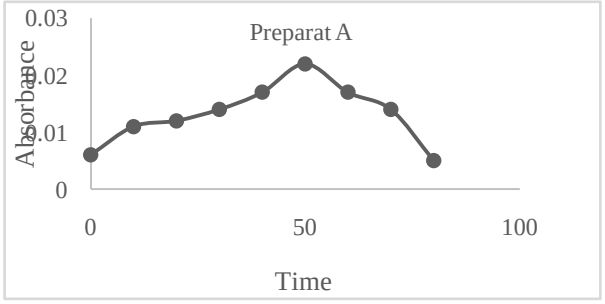
Measurement of size and distribution of nanoparticles using preparations that have good percent EE and in vitro release values. The results obtained in preparation A showed a particle size distribution of 786.6 nm with an average size of nanoparticles produced 10.6 nm (see appendix 12), which is in the range of size of the nanoparticles (10-1000 nm).

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.

***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)



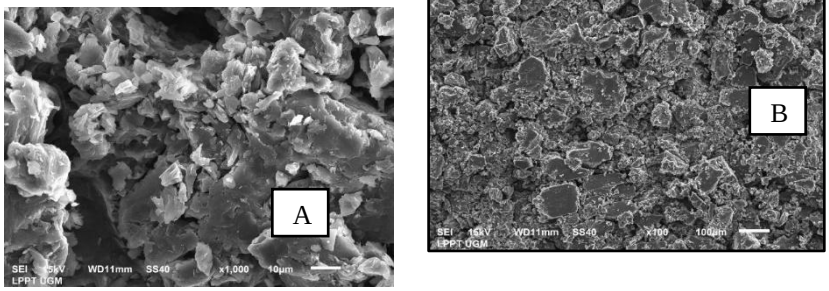


Figure 3. The micrograph of TQ-NP : A (3 mM), B (4 mM), C (5 mM) magnification 40 using an irmeco® microscope

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. Observations using SEMcan be seen in Figure 4. The results of observations on preparations A with a concentration of 3 mM showed a spherical shape on the surface of the particles.

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

TQ-NP *In Vitro Study*

To develop a nanoparticulate system, drug release and polymer biodegradation are important consideration factors. In general, the level of drug release depends on: (1) drug solubility; (2) surface desorption from adsorbed/ bound drugs; (3) drug diffusion through the nanoparticle matrix; (4) the erosion / degradation of the nanoparticle matrix and (5) the combination of the erosion / diffusion process.Solubility, diffusion and biodegradation of matrix materials regulate the release process[15, 22,24,.]

The nanoparticle diffusion release test was carried out using a franz diffusion cell withphosphate 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. The release of the drug can be caused by the presence of drugs that are on the surface of the nanoparticles.

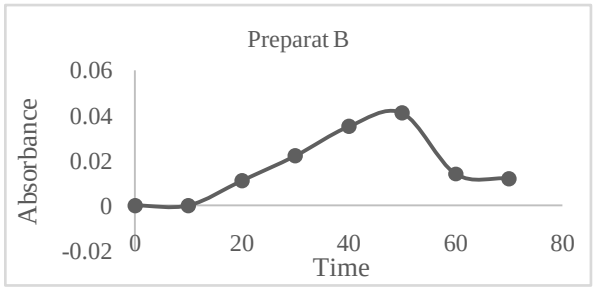


Figure 3. In-vitro release graphics for preparation A with a concentration of 3 mM

The results of the studies in Figures 7, 8, and 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 (can be seen in figures 4 and 5).

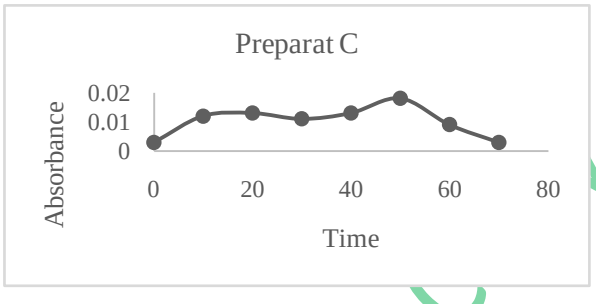
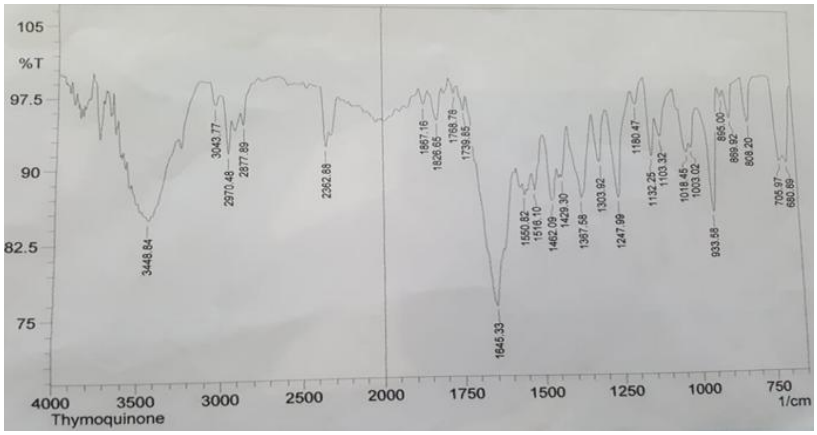


Figure 4. In-vitro release graphs of the 4 mM B preparations

Figure 5. In-vitro release graphics for C preparations with a concentration of 5 mM

The results showed that in Figure 4 and 5 the highest drug release occurred in the 50th minute and no release occurred in the 70th minute.

FT-IR Analysis



FT-IR or Fourier Transform-Infrared Spectroscopy measurements were used to determine the functional groups of nanoparticles and to determine the interactions that occurred between the drug ingredients, PEG 6000, with the wave number formed.

Figure 6. Results of FT-IR testing on thymoquinone

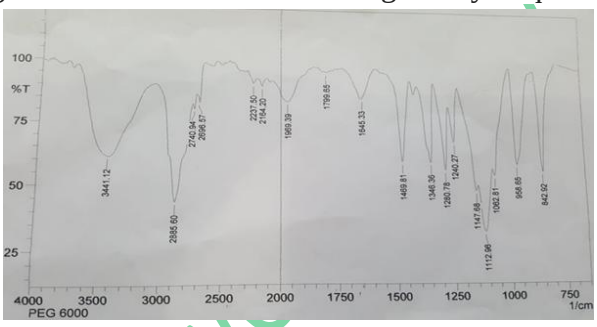


Figure 7. Results of FT-IR testing on PEG 6000

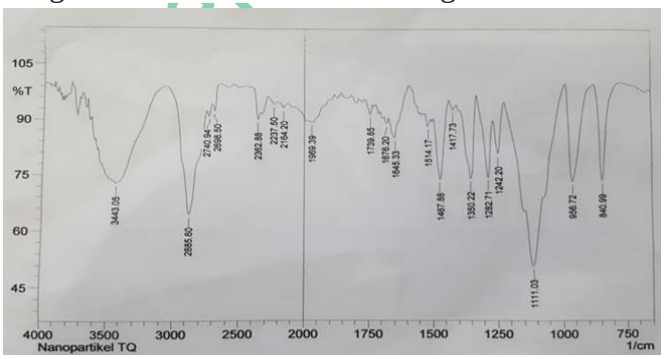


Figure 8. Results of FT-IR testing on thymoquinone nanoparticles

The FT-IR results shown in Figures 10, 11, 12 show similarities between thymoquinone nanoparticles and PEG 6000. This means that there is no chemical interaction between the polymer and the medicinal ingredients. After being characterized using FT-IR we found similar wave numbers from the drug coated with absorbing PEG.

CONCLUSION

From the results of this study Thymoquinone can be prepared into nanoparticles using Polyethylene Glycol 6000 with a concentration of 3 mM, 4 mM, and 5 mM. 99.9628 ± 0.026, and 99.9363 ± 0.049 respectively.

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