RESEARCH PAPER

Evaluating the potential of polymer-based nanoparticles in the delivery of thymoquinone: implications for bioavailability and drug efficacy

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ABSTRACT

Nigella sativa, commonly known as black cumin, kalonji, or kalajeera, belongs to the Ranunculaceae family and is renowned for its diverse therapeutic applications in conditions such as asthma, diarrhea, and dyslipidemia. This study focuses on thymoquinone, the primary bioactive compound in Nigella sativa, chemically characterized as 2-isopropyl-5-methylbenzo-1,4-quinone (C10H12O2). Thymoquinone exhibits various pharmacological activities, including anti-diabetic, anti-inflammatory, hepatoprotective, antimicrobial, antioxidant, and cardiac stimulant effects. We have investigated various nanoparticle formulation techniques, such as solvent evaporation, salting-out, emulsification, and nanoprecipitation, to develop polymeric nanoparticles to enhance thymoquinone delivery. Special attention was given to thymoquinone-loaded PLGA nanoparticles, which demonstrated significant antimicrobial and antioxidant properties, and to chitosan-based nanoparticles prepared via ionic gelation. Our findings indicate that these polymeric nanoparticles hold considerable promise for targeted and effective drug delivery, potentially revolutionizing the treatment of complex diseases. The study highlights critical methods and characterization techniques, including morphology, zeta potential, chemical composition, particle size distribution, and in vitro release kinetics, which are essential for optimizing the therapeutic efficacy of nanoparticle formulations.

Keywords: *Thymoquinone, Nigella sativa, Nanoparticles, Evaluation parameters, Chemical composition.*

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INTRODUCTION

Nigella sativa, commonly known as Kalonji, Kalajeera, or black cumin, belongs to the Ranunculaceae family [1-2]. It contains thymoquinone (TQ), a potent bioactive phytochemical compound that exhibits a variety of biological activities, including anticancer, anti-inflammatory, anti-diabetic, antioxidant, anti-ulcerative, antimicrobial, and immunomodulatory effects. In addition to TQ, Nigella sativa contains other bioactive constituents such as thymohydroquinone, di-thymoquinone, trans-retinol, and tocopherols [3]. The plant is also traditionally used as an emmenagogue, digestive aid, liver tonic, appetite stimulant, and antidiarrheal, and is believed to enhance milk supply in lactating mothers. In recent decades, there has been growing interest in utilizing phytochemicals as nutraceutical agents in pharmaceutical and food formulations [4]. Despite its potent therapeutic potential, TQ's efficacy and oral bioavailability are limited due to its poor solubility and formulation challenges. Recent academic efforts have focused on improving the nanoencapsulation techniques for poorly bioavailable drugs and phytochemicals [5]. The design of the carrier system plays a critical role in enhancing the pharmacodynamics and pharmacokinetics of bioactive compounds. Poly(lactic-co-glycolic acid) (PLGA), a biocompatible and biodegradable copolymer, has been widely used to improve the oral bioavailability of many bioactive substances [6]. In the body, PLGA degrades into glycolic acid and non-toxic lactic acid, and studies have demonstrated that PLGA is non-toxic based on animal models and cell culture studies [7]. In this study, thymoquinone-loaded PLGA nanoparticles were designed using polyvinyl alcohol (PVA) as a stabilizing agent. The nanoformulation was characterized by particle size, encapsulation efficiency, in vitro release profile, and antimicrobial and antioxidant activities. The review also outlines the techniques and methods used to prepare thymoquinone nanoparticles.

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Chemistry of thymoquinone

Chemically, thymoquinone is known as 2-isopropyl-5-methylbenzo-1,4-quinone. It is a dark yellow crystalline compound with a molecular weight of 164 g/mol, and a monoterpene diketone characterizes its structure. Due to its low molecular weight and a Log P value (less than five), thymoquinone can cross the blood-brain barrier [8]. Structurally, thymoquinone shares similarities with coenzyme Q, which plays an essential role as an antioxidant. Oxidation of thymol with hydrogen peroxide results in the formation of thymoquinone in gram quantities [9]. According to a proposed hypothesis, thermal processing of thymoquinone induces its oxidation, leading to the formation of a more potent compound [10]. The oxidation of thymol first produces thymo-hydroquinone, which is subsequently converted into thymoquinone. Over time, accumulating these intermediates leads to a significant yield of thymoquinone. Additionally, photoisomerization can lead to the formation of a dithymoquinone dimer [11-12].

Preparation of nanoparticles using different polymers

Natural polymers

Natural polymers are biopolymers derived from plant sources [13, 14]. Albumin, gelatin, sodium alginate, and chitosan are commonly utilized polymers for nanoparticle fabrication.

Synthetic polymers

Synthetic polymers are derived from petroleum-based sources [15-25]. Commonly used synthetic polymers for nanoparticle fabrication include poly(methyl methacrylate), poly(ethylene glycol), polycyanoacrylates, polyanhydrides, polyglycolides (PGA), polylactides (PLA), and poly(lactide-co-glycolide) (PLGA). Other examples include poly(N-vinyl pyrrolidone), poly(vinyl alcohol), poly(acrylic acid), polyacrylamide, polyorthoesters, polycaprolactone, polyglutamic acid, polymalic acid, and poly(methacrylic acid).

Generally, polymers can be categorized into two broad types, as detailed in the following table:

Table 1. Different polymers used for the formulation of TQ- nanoparticles

Type of Polymer	Polymer used	Remarks	Purpose	Reference
Natural polymers	Chitosan	Hydrophilic L-ascorbic acid and hydrophobic TQ together show that a wide range of highly effective drugs with low systemic absorption might be co-encapsulated in NP systems to boost their therapeutic efficacy.	As an effective antioxidant	[26]
	Albumin	The albumin nanoparticles loaded with TQ contain an average size of 315.6 nm. The overall zeta potential of TQ-loaded nanoparticles was measured at +6.79 mV. The findings indicated that TQ had an impact on planarian behavior, particularly on head regeneration.	To determine the effect of thymoquinone on neural regeneration	[27]
Synthetic polymers	Zinc oxide	The findings highlight the effectiveness of co-therapy with low-dose ZnO NPs and TQ (Group V), which is superior to utilizing low-dose ZnO NPs (Group III) or TQ alone. It is also as effective as a high dose of ZnO NPs (Group IV).	To evaluate the effectiveness of thymoquinone (TQ) alone or in combination with zinc oxide nanoparticles (ZnO NPs) as a therapeutic agent.	[28]
	Zinc oxide	The findings showed that TQ-ZnO nanoparticles enhanced DNA damage and suppressed cell growth during the synthesis phase, both of which contributed to apoptosis.	To determine the effect of TQ-NP on TNBC cells	[29]
	AgNP	TQ co-administration reduced the levels of pro-inflammatory mediators in the liver and kidney, including NF-κB, TNF-α, TGF-β, and IL-1β.	Thymoquinone's (TQ) ability to show protective action in rats from AgNP-induced hepatic and renal cytotoxicity	[30]
	Mesoporous silica	The distribution of TQ showed that loading TQ on MSNPs changed the way the protein was distributed across the brain, giving the cortex, thalamus, midbrain, and hypothalamus more access to drugs.	Targeting different brain areas	[31]

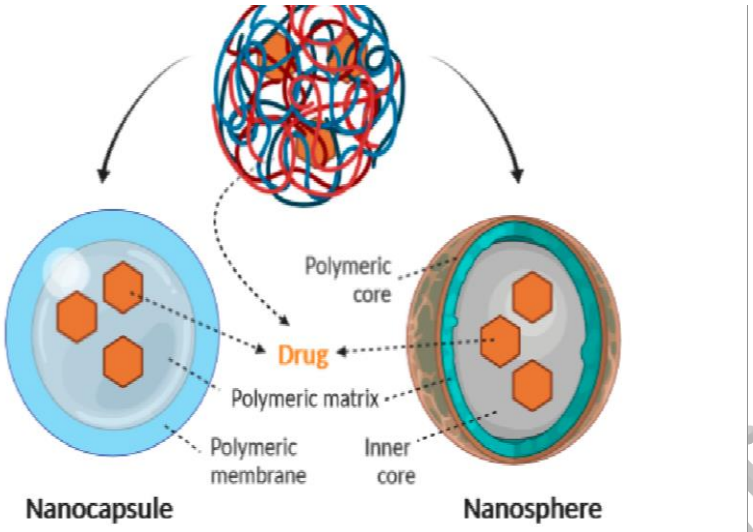

Fig. 1. Schematic representation of the nanocapsule and nanosphere structure [37]

Various methods for the formulation of polymeric nanoparticles

Various methods are employed to prepare polymeric nanoparticles, including nanospheres and nanocapsules. These techniques include solvent evaporation, salting-out, emulsification, nanoprecipitation, and others. The choice of polymer often dictates the preferred method for nanoparticle production. However, using solvents in these processes can pose challenges, leading to potential toxicity and environmental risks. Despite this, these methods typically generate aqueous colloidal suspensions of nanoparticles [32-36]. The two types of polymeric nanoparticles—nanocapsules and nanospheres—are generally recognized as reservoir systems. Figure 1 illustrates the nanocapsule and matrix (nanosphere) systems [37].

Solvent evaporation techniques

This method forms an oil-in-water emulsion using an organic solvent, producing nanospheres, as illustrated in Figure 2. However, solvents such as dichloromethane and chloroform are known to be toxic and have been replaced by ethyl acetate, which demonstrates better biological compatibility [38, 39]. The organic solution is then emulsified using the appropriate surfactants via high-speed homogenization or ultrasonication to form nanodroplets. These nanoparticle suspensions are further processed by solvent evaporation under continuous magnetic stirring while maintaining a proper room temperature. Finally, the nanoparticles are washed, centrifuged, and freeze-dried, forming nanospheres [40-44].

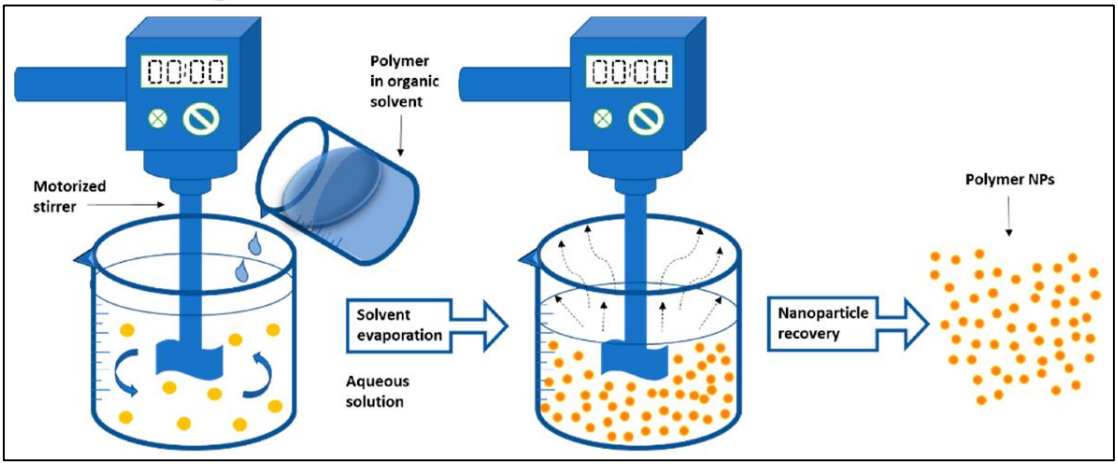

Fig. 2. Solvent-evaporation Technique [45]

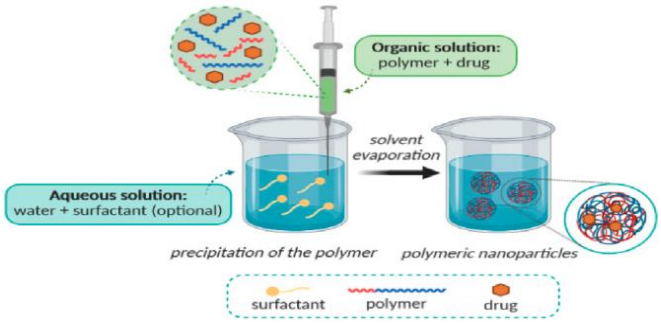

Fig. 3. Diagrammatic representation for the preparation of nanoprecipitation method [37]

Nanoprecipitation

Nanoprecipitation involves the precipitation of a preformed polymer from an organic solution, followed by the diffusion of the organic solvent into an aqueous medium, either with or without the presence of a surfactant, as illustrated in Figure 3. Poly(lactic acid) (PLA) is a polymer that can be dissolved in a water-miscible solution of intermediate polarity, leading to the precipitation of nanospheres. In the aqueous phase, a stabilizer or surfactant is introduced, which facilitates the deposition of the polymer at the interface between the organic solvent and the water. This process occurs due to the rapid diffusion of the solvent, resulting in the instantaneous formation of a colloidal suspension [46-48].

Phase separation is initiated using a completely miscible solvent that is also a non-solvent for the polymer, promoting the formation of colloidal polymer particles during the initial stage of the process [49]. The solvent displacement method enables the creation of nanocapsules by introducing a small amount of non-toxic oil into the organic phase. This oil fills the central core of the nanocapsules, resulting in high loading efficiency, particularly for lipophilic drugs, during the nanocapsule preparation process.

This simple method can be applied to water-miscible solvents, where emulsification occurs due to a sufficiently high diffusion rate. Although the process is spontaneous, the solubility of water-miscible solvents can lead to instability if the rate of droplet coalescence is high. While solvents such as acetone and dichloromethane (ICH Class 2) commonly dissolve the polymer and increase drug encapsulation efficiency, dichloromethane can increase the mean particle size and is considered toxic [50].

Lipophilic drugs benefit most from this method due to their solubility in the aqueous phase, making it unsuitable for encapsulating water-soluble compounds. Various polymeric materials, such as PLGA [36], PLA [43], PCL [44], and poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA), have been incorporated into this process [51-56]. Trapping efficiencies as high as 98% have been achieved, making this method suitable for incorporating cyclosporin A [47]. To facilitate the parenteral administration of poorly soluble antifungal drugs like bifonazole and clotrimazole, highly loaded nanoparticulate systems based on amphiphilic β-cyclodextrins were prepared using the solvent displacement process [57, 58].

Emulsification or Solvent Diffusion (ESD)

This procedure typically results in the formation of an oil-in-water (o/w) emulsion. The encapsulating polymer, drug, and solvents, such as ethyl acetate and benzyl alcohol, are present in the emulsion phase and are subsequently diluted with water, as shown in Figure 4.

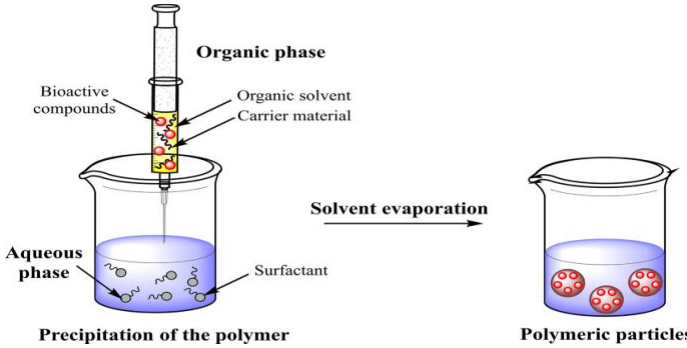

Fig. 4. Schematic representation of the nanoprecipitation technique [63]

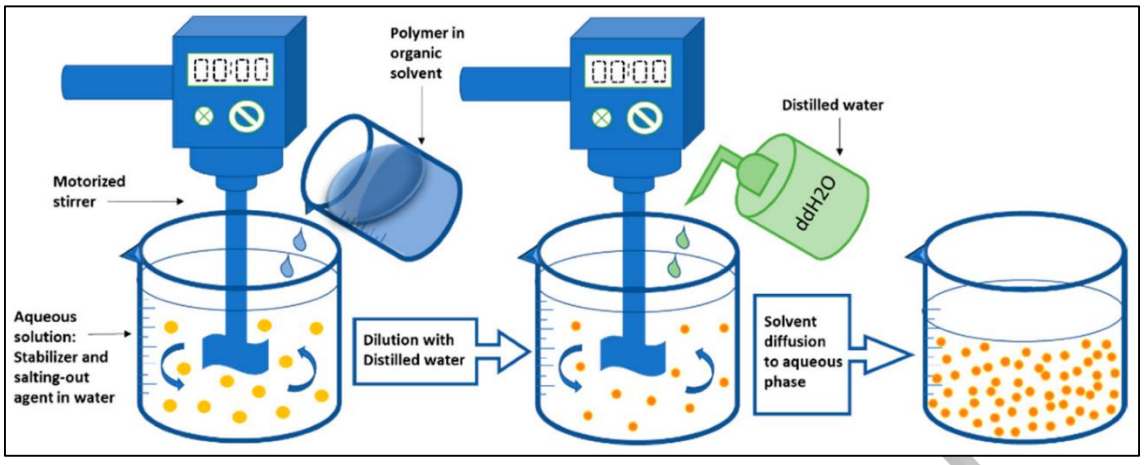

Fig. 5. Schematic representation of the Salting out technique [45]

To maintain thermodynamic balance at room temperature [59], large volumes of water are added to dilute the system, promoting the diffusion of dispersed droplet particles into the outer phase, leading to nanospheres and nanocapsules. Nanocapsules can be produced by adding small droplets of triglycerides in the appropriate ratio to the polymer [60]. The solvent is then evaporated using filtration and evaporation methods, which depend on the solvent's boiling point. The resulting nanoparticle dimensions typically range from 80 to 900 nm. These techniques offer advantages such as high batch-to-batch reproducibility, high encapsulation efficiency, narrow size distribution, ease of scale-up, and simplicity [61, 62].

Emulsification or reverse salting-out

This method, known as the salting-out technique, is illustrated in Figure 5. It is primarily based on separating hydrosoluble solvents from an aqueous solution [64]. The formation of an oil-in-water (o/w) emulsion in this method varies in composition, typically involving water-soluble polymer solvents like ethanol and acetone, along with salting-out agents such as magnesium chloride, magnesium acetate, calcium chloride, and non-electrolytes like sucrose. Colloidal stabilizers, including hydroxyethyl cellulose and polyvinylpyrrolidone, are also used to form an aqueous gel [65].

The preformulated oil-in-water (o/w) emulsion is diluted with deionized water, promoting diffusion into the external phase, forming nanospheres. Cross-flow filtration is then used to eliminate the remaining solvent and salting-out agents [66]. The choice of salting-out agents plays a critical role in enhancing encapsulation efficiency during the production of nanospheres. The size of the nanospheres typically ranges from 170 to 900 nm, with the final size adjusted between 200 and 500 nm, depending on the polymer concentration in the internal phase relative to the volume of the external phase [67].

Dialysis

The dialysis method is well-known for preparing narrow and uniformly dispersed nanoparticles. The solvent and polymer are combined in this process, with the polymer's molecular weight maintained within the dialysis tube. Dialysis is performed against non-miscible solvents, using a miscible solvent [68]. Within the membrane, the loss of solubility occurs due to polymer aggregation through the solvent displacement method, leading to a homogeneous nanoparticle suspension formation. This technique has successfully prepared various copolymers and several polymers [69]. For example, using dimethylformamide (DMF) as a solvent, nanoparticles such as poly(lactide)-b-poly(ethylene oxide) and poly(benzyl-l-glutamate)-b-poly(ethylene oxide) were prepared. The polymeric solution in this process influences the particle size distribution and morphology of the nanoparticles [70]. The choice of solvent used during preparation significantly impacts the nanoparticles' morphology and particle size [71-77]. A novel process based on osmosis for preparing different types of polymeric nanoparticles, both natural and synthetic, is illustrated in Figure 6.

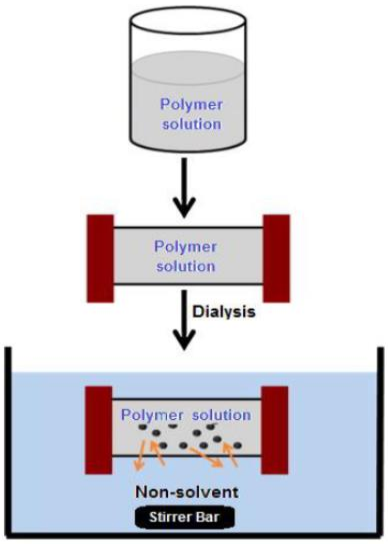

Fig. 6. Diagrammatic illustration of the osmosis-based polymer nanoparticle preparation process [78].

Table 2. Different methods used in the Fabrication of TQ-nanoparticles

Type of Nanoparticle	Procedure	Technique used	Results	Reference
Chitosan (CS) NPs	The ionic gelation procedure was followed in the preparation of CS NPs. After adding TPP (tripolyphosphate) aqueous solution dropwise to a CS solution while stirring continuously at room temperature, placebo NPs were produced. The (+) charged amino groups of CS and the (-) groups of TPP interacted ionically to generate NPs. The investigations were used to determine the CS/TPP ratio.	Ionic gelation	The particle diameter of 150–200 nanometers is validated and can be characterized through transmission electron microscopy and dynamic laser light scattering. The findings demonstrated that the drug: chitosan ratio had a substantial impact on the formulation's particle size.	[79]
Chitosan (C) modified polycaprolactone (PL) nanoparticles (NPs)	At 800 revolutions per minute, the aqueous phase was continuously stirred while the organic phase was introduced dropwise. Finally, to obtain nanosized TQ, the resulting dispersion was sonicated for three minutes.-CPLNPs	single emulsion solvent evaporation	Several parameters were used to characterize the optimized THQ-CPLNPs. The size, PDI, and ZP of THQ-CPLNPs were found to be 182.32±6.46 nm, 0.179±0.012, and 21.36±1.22 mV, in that order.	[80]
PLGA NPs	Using a probe sonicator, the oil and aqueous phases were combined to create an emulsion. This was then gradually poured into a 24 mL water dispersion medium and stirred magnetically for two hours to ensure all of the solvents had evaporated.	solvent evaporation	After dissolving TQ(10 mg) and PLGA(60 mg) in 2 mL in the ratio 20:80 v/v mixture of ethyl acetate (EA) and dichloromethane (DCM), the aqueous phase was added to the oil phase. Using a probe sonicator, the oil and aqueous phases were combined to create an emulsion. This was then gradually poured into a 24 mL water dispersion medium and stirred magnetically for two hours to ensure all of the solvents had evaporated.	[81]
TQ-NP	In 0.1 ml of DMSO, PLGA(65 mg) of Pluronic F-127(20 mg) and TQ (15 mg) used to be dissolved while being stirred for 30 minutes. After that, the entire solution was emulsified for a further ninety seconds using 0.7 ml of 2% PVA and probe sonication. The second emulsion was then formed by sonicating the emulsion for 60 seconds with 0.2 ml of 1% chitosan. Centrifugation was used twice to wash the TQ-NPs with deionized	double emulsion method	In HCT116 cells, TQ and TQ + DOX raised Bax levels, while in MDA-MB-231-Luc cells, Bcl2 levels were lowered. When combined with DOX, the TQ-NPs, which had an average size of 218 nm, dramatically decreased the size of the tumor in the xenograft model.	[82]

Type of Nanoparticle	Procedure	Technique used	Results	Reference
	water for 60 min). They mixed the residue product with 1 ml of chitosan.			
THQ-loaded lipid-polymer hybrid nanoparticles (THQ-LPHNPs)	CHS (50–90 mg) was added to 0.1% acetic acid solution(15 mg) to create the aqueous phase, which was then properly dissolved at room temperature. The aqueous phase was then supplemented with a surface active agent (P-188; 75–125 mg) and gently stirred to further dissolve it. The organic solution was then created by adding precisely weighed THQ (10 mg) and lipid (PL-90G; 100–150 mg) to 2 mL of N, N-dimethylformamide. The mixture was then gently stirred at a speed of 850 rpm at room temperature until the lipid was thoroughly dissolved.	Single-step nanoprecipitation method	NPs had a low PDI value (0.217 ± 0.013), a positive high zeta potential (>25 mV), and a nano-metric size of 179.63 ± 4.77 nm. 4.7 times more bioavailability was shown by TQ-LPHNPs than by THQ suspension.	[83]
Thymoquinone-PLGA-PVA nanoparticles (TQ-PLGA-PVA-NPs)	To obtain a homogeneous solution, PLGA powder (80 mg)was dissolved in 2 mL of HPLC-grade dichloromethane for 12 hours at room temperature in an oil phase. The solution then received the addition of TQ(50 mg) To create the solid/oil primary emulsion, the suspension was subjected to two minutes of sonication using sonication equipment. The mixture (TQ/PLGA) was then rotated at 400 rpm in a magnetic stirrer to create a solid/oil/water emulsion. This was achieved by emulsifying aqueous phase of saline (20 mL)with PVA 1% w/v. To create the final emulsion, the suspension was ultra-sonicated (20 KH2) for three minutes.	Modified emulsion technique	A promising combination, TQ loaded PLGA-PVA nanoparticles may be employed to reduce bleomycin-induced pulmonary fibrosis by downregulating iNOS in lung tissue and regulating TGF-β1 and IL 10.	[84]

Procedure for the formulation PLGA-TQ nanoparticles

Thymoquinone nanoparticles were formulated using the evaporation and diffusion method with poly (lactic-co-glycolic acid) (PLGA) as the polymer. A 45 mg quantity of polymer was dissolved in 2 mL of HPLC-grade dichloromethane at room temperature for 4 hours to obtain a uniform solution. This method solubilized the lipophilic compound in the chosen solvent. The suspension was then sonicated for at least 2 minutes to form the primary oil-in-water (S/O) emulsion. The solution was emulsified with 20 mL of polyvinyl alcohol (PVA) acting as the aqueous phase, under continuous stirring at 400 rpm. The suspension was vortexed at high speed for at least 10 seconds, followed by a second round of sonication for 3 minutes to obtain the final emulsion. The remaining solvent in the suspension was evaporated using a rotary evaporator at 60°C under vacuum. Finally, the suspension was centrifuged for 20 minutes at 4°C, resuspended in a cryoprotectant solution, and lyophilized to obtain the nanoparticles [85].

Methods involved in chitosan-based nanoparticles

Nanoparticles based on chitosan were formulated using the ionic gelation method. Placebo nanoparticles were prepared by dissolving tripolyphosphate in an aqueous solution, followed by continuous stirring using a magnetic stirrer at room temperature [86]. The nanoparticles were formed by interacting with the negatively charged tripolyphosphate and the positively charged chitosan solution. Thymoquinone-loaded nanoparticles were investigated for in vitro release profiles and initial thymoquinone concentration. The nanoparticle sample was subsequently centrifuged for 30 minutes at 15,000 rpm at 4°C to separate the nanoparticles [87].

Characterization of polymeric nanoparticles

Morphology

Electron microscopy techniques, such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), are commonly used to analyze the size and morphology

of nanoparticles. Both SEM and TEM have been widely recognized for characterizing polymeric nanoparticles. Typically, these techniques are combined with cryofracture methods to examine nanoparticle morphology [88]. TEM is beneficial for distinguishing between the size and shape of nanocapsules and nanospheres, where nanospheres are typically spherical, and nanocapsules have thicker walls. Additionally, techniques such as atomic force microscopy (AFM) are extensively used to characterize surface morphology [89]. These methods provide high-resolution 3D imaging and detailed surface information at the atomic level. AFM can also reveal surface features such as small cavities on the nanoparticle topography [90].

Particle size

The size of nanoparticles can be measured using various techniques, including dynamic light scattering (DLS), static light scattering (SLS), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). However, the latter two are less commonly used. Since these techniques differ, the measured size can vary depending on the method employed. Electron microscopy generally provides images of particles isolated from their surroundings [91]. In contrast, DLS includes information about the radius of suspended particles and is particularly useful for measuring the size of aggregated nanoparticles in solution [92]. Typically, polymeric nanoparticles have a mean diameter ranging from 100 to 300 nm, with particle sizes of 60 to 70 nm, or smaller, often considered ideal for nanoparticle formulations [93].

Crystal structure and chemical composition

The chemical composition of nanoparticles can be determined using atomic absorption spectroscopy (AAS), which operates on the principle of atomic absorption. In this technique, atoms transition from the ground to the excited state by absorbing energy at a specific wavelength [94]. Another helpful technique, mass spectrometry, aids in identifying the composition of the nanoparticle. In this method, analytes are ionized into a gaseous state with minimal fragmentation, and the separation of the ions or atoms is detected using a time-of-flight mass analyzer. This analyzer sorts particles based on their mass-to-charge ratio [95]. Additionally, the structural arrangement of nanoparticles, whether in amorphous or crystalline form, can be characterized using X-ray diffraction (XRD) analysis, typically applied to nanoparticles in powdered form [96-98].

Zeta potential

Doppler techniques can be employed to measure the velocity of charged particles in a solvent and to determine the zeta potential. The zeta potential is calculated using electrophoresis, which involves measuring the mobility of charged particles [99]. Major components such as poloxamers, polymers, and phospholipids are commonly used to assess the zeta potential of nanoparticles. A high zeta potential of 30 mV or greater is desired for optimal physicochemical stability in colloidal suspensions, as the repulsive forces prevent nanoparticle aggregation due to collisions with adjacent particles [100]. The zeta potential reflects the charge on the particle surface, which is influenced by changes at the interface between the dispersion medium and the adsorption of ionic species in the aqueous phase [101-103].

Pharmaceutical in vitro release kinetics

Studies have indicated that the drug release pattern is generally governed by either the desorption of the drug from the particle surface, the erosion of the polymeric matrix, or the diffusion of the drug through the nanosphere matrix [104]. Kinetic studies of drug release from nanospheres suggest that the release follows first-order kinetics, driven by the diffusion of the drug through the polymeric matrix [105]. In the case of nanocapsules, the drug release kinetics typically follow zero-order kinetics, where the drug is dissolved in the oil phase. The release from the vesicular structure is then dependent on the diffusion of the drug through the polymeric wall [106-108].

The study highlights that nanoparticles with sizes around 100-300 nm can efficiently penetrate tissues and offer higher bioavailability compared to larger particles. The zeta potential (positive or negative charge) plays a crucial role in the stability of nanoparticles in biological fluids, as a high zeta potential prevents aggregation by ensuring repulsion between particles, thus maintaining bioavailability and efficacy.

PLGA, being biodegradable, gradually releases thymoquinone, making it suitable for sustained-release therapies. In contrast, chitosan enhances mucoadhesion, which is particularly useful for formulations targeting mucosal tissues, such as nasal or oral delivery systems.

Future direction blocking the clinical aspects

Thymoquinone (TQ) formulation offers a cost-effective and straightforward approach to achieving controlled water solubility and stability while enhancing the effective delivery of TQ across

therapeutic targets. However, challenges remain in translating nanoparticle-based TQ formulations into clinical practice. Early problem-solving during formulation development is critical for the success of nano-TQ delivery systems. This underscores the potential benefits of a prodrug strategy to optimize TQ's pharmacological effects. Encapsulating TQ in a nanoformulation can alter its structure, improving its drug-like properties. When developing nano-TQ delivery systems, the excipient must be highly biocompatible, biodegradable, and easy to formulate. Since excipients are typically used in large quantities in nano-TQ formulations, they should not introduce negative side effects. Nano-TQ formulations should effectively deliver sufficient TQ to the target area of the disease. In other words, the success of nano-TQ relies on achieving high TQ loading and using specific transport channels. As a preliminary step, compatibility between TQ and the excipient should be thoroughly evaluated.

Controlling batch-to-batch reproducibility is relatively straightforward at small scales but becomes more challenging at larger or industrial scales. To address this, techniques such as spray drying and microfluidics have been explored to facilitate the effective clinical translation of nanomedicines. Additionally, TQ's poor aqueous stability may pose significant challenges during the nanomanufacturing process, potentially affecting lyophilization, sterilization, and/or purification of nano-TQ. Strong collaborations between academic research labs and pharmaceutical companies are essential to bridge the gap in clinical translation and overcome these challenges.

CONCLUSION

The review focuses on evaluating the most effective techniques for formulating nanoparticles. Several methods are available, but only the most suitable ones are selected to design nanoparticles with the desired size and efficient thymoquinone entrapment. Recent advancements in the preparation of polymer-based nanoparticles have been explored, demonstrating promising pharmacological activities. Significant progress has been made in understanding the physicochemical properties that contribute to developing more stable polymeric nanoparticle formulations, which may enhance their clinical applications. Looking ahead, it is crucial to obtain both quantitative and qualitative data to support the establishment of standardized techniques, guidelines, and protocols, ensuring the reliability and quality of analytical results.

CONFLICTS OF INTEREST

There is no conflict of interest.

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AUTHOR CONTRIBUTION

Sweety Saini: Conceptualization, Writing-original draft. Ali Sartaj: Editing, Formal Analysis, All investigation. Aashish: Formal Analysis.

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