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# Formulation and characterization of berberine loaded zein nanoparticles

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## Abstract

The objective of the present investigation is to formulate zein nanoparticles and encapsulate it with phytoconstitutent berberine and characterize the nanoparticles for various parameters. the zein nanoparticles were optimized by nanoprecipitation method. Various ratio of zein-guargum (10:1, 5:1, 1:1, 1:2 and 1:5) were used to optimize the ratio for NP preparation. The stirring time, stirring speed and the pH of nanoprecipitation was also optimized. The optimized conditions included 1:2 zein-guar gum ratio, stirring speed 600 rpm for 30 min and nanoprecipitation pH of 4.0. Berberine was loaded at three mass ratios with respect to zein (20:1, 10:1 and 5:1) for nanoprecipitation. The berberine loaded nanoparticles were evaluated for production yield, encapsulation efficiency, particle size, polydispersity, zeta potential, *in vitro* drug release by dialysis method and antioxidant activity by DPPH assay. The average particle size of the blank zein nanoparticles was obtained by DLS to be 160.4 nm with a zeta potential of -17.3 mV. The yield of the berberine loaded nanoparticles was found to be 83.2-91.5% whereas the encapsulation ranged from 45.3 to 69.7%. The particle size was found to be 191.5 nm to 251.8 nm with polydispersity index ranging from 0.418 to 0.701. A maximum of 69.18% berberine was released in 4 hours. Berberine has an elimination half-life of around 4.8 hours and the decline in cumulative drug percentage may be due to the degradation of berberine in solution after 4 hours. The $IC_{50}$ value was calculated from the plot and was found to be 122.058 µg/mL and 98.984 µg/mL for berberine and the berberine loaded zein nanoparticle respectively.

## Keywords

Berberine, antioxidant, nanoprecipitation, zein, nanoparticles

## Introduction

Nanotechnology has changed the world itself in all aspects, a large scale development taken place in biomedical fields. Nanoparticle mediated drug delivery has tremendous applications than the conventional methods because its property of controlled release of drug at specific sites. Nanoparticle can also protect the drug from rapid degradation and improves intracellular penetration of drugs (Nasrollahzadeh et al., 2019). Drug delivery using nanoparticles have a lot of advantages like longer retention of drug in the blood stream, less toxicity and side effects, site specific targeting etc (Liu et al., 2008). Zein nanoparticles were successfully applied as a carrier for controlled release of drug and dietary supplements because of its inherent biodegradability and biocompatibility (Luo and Wang, 2014).

Zein nanoparticles as drug delivery vehicles offer a multitude of advantages, including increased oral bioavailability of drugs and reduced dosage frequency. A variety of anticancer, antimicrobial, antituberculosis, peptide and protein based drug formulations with nano drug carriers have been researched for their therapeutic effectiveness (Chen et al., 2024; Li et al., 2023; Gali et al., 2022; Panagiotopoulou et al., 2022; Calliari et al., 2020; Yoosaf et al., 2019). Inspite of the wide spread research, sustained release formulations with nanopolymeric carriers could be found only in small numbers in the market, with most of them in the form of ointments or wound healing bandages. Berberine is an alkaloid from *Hydrastis canadensis* L., Berberidaceae also found in many other plants. It is relatively toxic parenterally, but has been used orally for various parasitic and fungal infections and as antidiarrheal (drugbank, 2024; pubchem, 2024). It exhibits limited aqueous solubility, limited bioavailability and is efficacious as antioxidant compound. The objective of the present investigation is to formulate zein nanoparticles and encapsulate it with phytoconstitutent berberine and characterize the nanoparticles for various parameters.

## Material and Methods

### Preformulation Studies

In order to perform the preformulation evaluation of the drug tests of identification such as physical appearance, melting point and FTIR spectroscopy were carried out. The solubility profile of drug

in various solvent systems, incompatibility study by FTIR, partition coefficient and quantitative estimation of drug was also studied (Khare et al., 2023).

**Preparation of zein nanoparticles**

In order to prepare the zein nanoparticles, pectin was used as the stabilizing agent. The nanoprecipitation method was used to form zein-guar gum nanoparticles in suspension (Donsì et al., 2017). Briefly, zein (2.5% wt) was dissolved in an ethanol aqueous solution at 80% by weight in ethanol. Guar gum (5.0%) was prepared in distilled water by stirring using magnetic stirrer for 5 h and stocked overnight at ambient temperature. 10 mL of zein solution was added drop-wise to 30 mL of guar gum solution under continuous stirring (600 rpm) for 30 min. Samples were subjected to ethanol elimination under reduced pressure at 30°C using a rotary evaporator. The eliminated volume (about 75% of the initial volume) was replaced by water. The different suspensions were adjusted to pH 4 and stocked at 4°C.

**Preparation of sesamol loaded zein nanoparticles**

The drug (berberine) was dissolved in 10 mL of the zein (2.5%) stock solution in ethanol to give mass ratios of zein:berberine of 20:1, 10:1, and 5:1. The solution was sonicated for 5 min and then added dropwise to 30 mL of guar gum aqueous solution (5.0%) under magnetic stirring (600 rpm) at room temperature for 30 min (Gali et al., 2022).

**Evaluation of the berberine loaded zein nanoparticles**

**Yield**

The nanoparticles were dried by lyophilization and the mass of obtained by weighing the dried particles. The yield was determined by dividing the mass used for preparation of particles by the dry weight of particles obtained.

**Encapsulation Efficiency**

The encapsulation efficiency of the different formulations was assessed by quantifying the free berberine in the aqueous phase and deducting the amount of the encapsulated berberine. Samples

were centrifuged at 14000 rpm for 20 min. The supernatant obtained was filtered through 0.2 µm filter. The free berberine were then determined (Davidov-Pardo et al., 2015) by diluting with acetonitrile and measuring the peak area at 263 nm using the conditions mentioned in calibration curve by HPLC.

$$\%\ Encapsulaton = \frac{Total\ berberine - Free\ berberine}{Total\ berberine} \times 100$$

**Particle size and polydispersity**

The particle size and distribution of the powder was measured by dynamic light scattering spectroscopy (DLS) using a Malvern laser particle size analyzer. The instrument was equipped with a He-Ne laser source (λ=633 nm) and at scattering angle of 1730. The dispersion concentration was around 0.1 g/L. The suspension was prepared by dispersing the powder in distilled water and treated for 6mins in an ultrasonic bath to obtain a well-dispersed suspension (Akbari et al., 2010; Mishra et al., 2023).

**Zeta Potential**

The zeta potential testing of the aqueous dispersion of the zein nanoparticles was performed using a Malvern Zetasizer Nano ZS instrument equipped with a HeNe laser operating at 632.8 nm and a scattering detector at 173 degrees.

***In vitro* drug release**

*In vitro* release of berberine from the formulation in comparison to berberine suspension were performed using USP II dissolution apparatus at 50 rpm and a temperature of 37 ± 0.5 °C, where an accurate amount of the nanoparticle formulation, equivalent to 10 mg berberine, and berberine suspension (10 mg berberine in phosphate buffer) were placed in dialysis membrane bag and immersed in the dissolution medium (Mishra et al., 2015). Drug release studies were conducted in 500 ml phosphate buffer pH 7.4 (Sayed et al., 2018). Samples of 2 ml were withdrawn from the release medium at (1, 2, 3, 4, 5, 6, 8, and 24 h) and replaced with an equal volume of fresh medium and berberine concentration in the withdrawn samples was determined by HPLC at 263 nm

detection wavelength for the elute. The study was performed in triplicate and the average berberine released at each time interval were calculated.

***In vitro* anti-oxidant activity**

Determination of DPPH radicals scavenging activity was performed by the previously reported method (Khan et al., 2012; Mishra et al., 2017). Separately, 1mM solution of DPPH and test solution (25-125 µg/mL) were prepared in ethanol. 1.5ml of the test solution was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

$$\text{DPPH scavenged (\%)} = \frac{(A_{con} - A_{test})}{A_{con}} \times 100$$

$A_{con}$ - is the absorbance of the control reaction

$A_{test}$ - is the absorbance in the presence of the test solution.

**Results and Discussion**

The pure drug (active pharmaceutical ingredient) berberine was purchased from Yucca enterprises, Mumbai and the sample was observed for its organoleptic characters. It was obtained as yellow powder with no odor and a bitter taste. It melted at 146-148°C and had a octanol-water partition coefficient of 1.4.

**Preparation of zein nanoparticles**

The zein nanoparticles were stabilized with guar gum and the particles were found to be around 150-200 nm in size with a spherical morphological character. The particles as seen in the photomicrograph by scanning electron microscopy revealed structurally dense spheres having smooth exterior and almost undeviating sizes (Figure 1a). The average particle size of the blank zein nanoparticles was obtained by DLS to be 160.4 nm (Figure 1b) with a zeta potential of -17.3 mV (Figure 1c).

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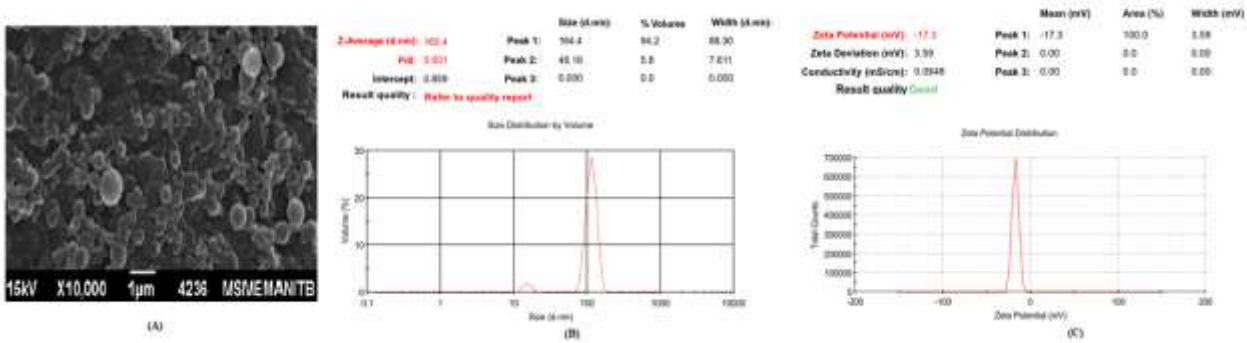


**Figure 1 (A) SEM image (B) Particle size (C) Zeta potential of blank zein nanoparticles**

**Optimization of zein nanoparticles**

Five ratio of zein to pectin were used for determining the optimum ratio resulting in stable nanoparticles. It was found that when the ratio of zein-guar gum was 10:1 and 5:1, the solution turned highly turbid on achieving pH 4.0 suggesting formation of very large particles. On the other hand reducing the zein-guar gum ratio to 1:1, resulted in small particles which accumulated rapidly on standing and 1:3 resulted in formation of large particles as indicated by a whitish solution formed on pH maintenance. On the other hand it was found that a 1:2 ratio of zein-guar gum resulted in small and stable particles as indicated by a bluish solution.

Since the 1:1 ratio of zein-guar gum resulted in small stable particles, this ratio was used keeping stirring speed and pH constant, varying the time of stirring. It was found that when the contents were stirred for 15 min, milky solution was formed suggesting large particles. On the other hand, an almost translucent, slighty whitish solution was obtained on stirring for both 30 and 45 min. Since increased stirring time did not result in significant change in particle size of the nanoprecipitate, a stirring time of 30 min was considered optimum.

Three stirring speeds were studied to optimize the speed required to produce stable nanoparticles. It was observed that at speed of 400 rpm, the precipitation of particles was not in the nano size range whereas at 600 and 8000 rpm, the particles produced were of almost similar size. Also it was found that the size distribution of the particles obtained at 800 rpm stirring was very high (0.813) as compared to that at 600 rpm (0.601). Hence a stirring speed of 600 rpm was considered as optimum to produce the most stable zein nanoparticles.

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The effect of pH of solution on nanoprecipitation was studied by carrying the process at four different pH. In highly acidic pH, neutral pH and alkaline pH the particles appeared unstable within a few minutes of storage and resulted in aggregation. At moderate pH of 3.0 and 4.0, the particles where small in size but at pH 3.0, they aggregated on standing displaying unstability while at pH 4.0 the particles were stable for longer duration of time and hence a pH of 4.0 was selected for optimized zein nanoparticles. Zein has an isoelectric pH of 6.2 and keeping pH below it helps in it retaining the positive charge on surface (Guo and Wang, 2014). Guar gum has negative charge and the formation of zein-guar gum complex results in an overall negative charge on the surface resulting in stable particles (Davidov-Pardo et al., 2015).

The most optimum conditions for nanoprecipition using zein and guar gum was scribed as zein-guar gum ratio of 1:2, stirring time 30 min at 600 rpm and pH of 4.0. These conditions were used to preparation of berberine loaded nanoparticles.

**Preparation and characterization of berberine loaded nanoparticles**

Berberine was added to the zein solution in three ratio 1:20, 1:10 and 1:5 and the nanoprecipitation was achieved using the optimized conditions. The particles obtained were evaluated for various parameters as per reported methods. The yield of the nanoparticles was found to be 83.2-91.5% whereas the encapsulation ranged from 45.3 to 69.7%. The particle size was found to be 191.5 nm to 251.8 nm with polydispersity index ranging from 0.418 to 0.701 (Figure 2a, 2b).

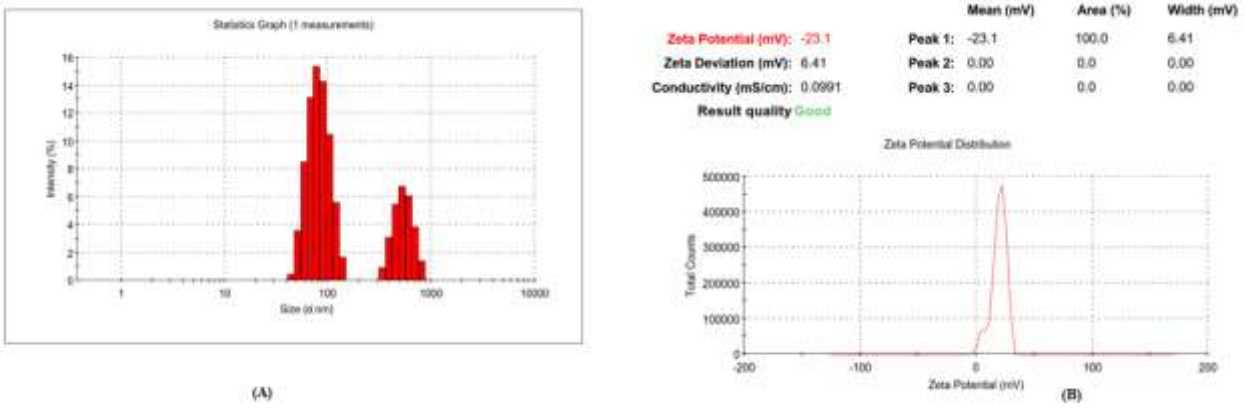


**Figure 2 Berberine zein (1:20) nanoparticle A) Particle (B) Zeta potential**

The results obtained for each berberine loaded zein nanoparticle formulation are presented in Table 1.

**Table 1 Evaluation of formed nanoparticles**

| S. No. | Berberine-zein ratio | Yield (%) | Particle size (nm) | PDI | Zeta Potential (mV) | %EE |
|---|---|---|---|---|---|---|
| 1 | 1:20 | 83.2 | 191.5 | 0.418 | -23.1 | 69.7 |
| 2 | 1:10 | 86.7 | 218.7 | 0.701 | -21.7 | 56.1 |
| 3 | 1:5 | 91.5 | 251.8 | 0.511 | -19.6 | 45.3 |

The highest entrapment and smallest particle size was obtained when 1:20 ratio of berberine-zein was used. Hence it was considered the best formulation and this formulation was used for studying the drug release and antioxidant activity.

***In vitro* release of berberine from zein nanoparticles**

The release of berberine was studied by dialysis method for a duration of 24 h in phosphate buffer. It was observed that the release of berberine from suspension increased rapidly for 4 hours and then it started to decline from the $5^{th}$ hour till the end of $24^{th}$ hour. A maximum of 69.18% berberine was released in 4 hours. Berberine has an elimination half-life of around 4.8 hours and the decline in cumulative drug percentage may be due to the degradation of berberine in solution after 4 hours. On the other hand, when encapsulated in zein nanoparticles, after an initial burst release of 10.19% in the first hour, 89.43% berberine was released steadily over 24 hours (Figure 3)

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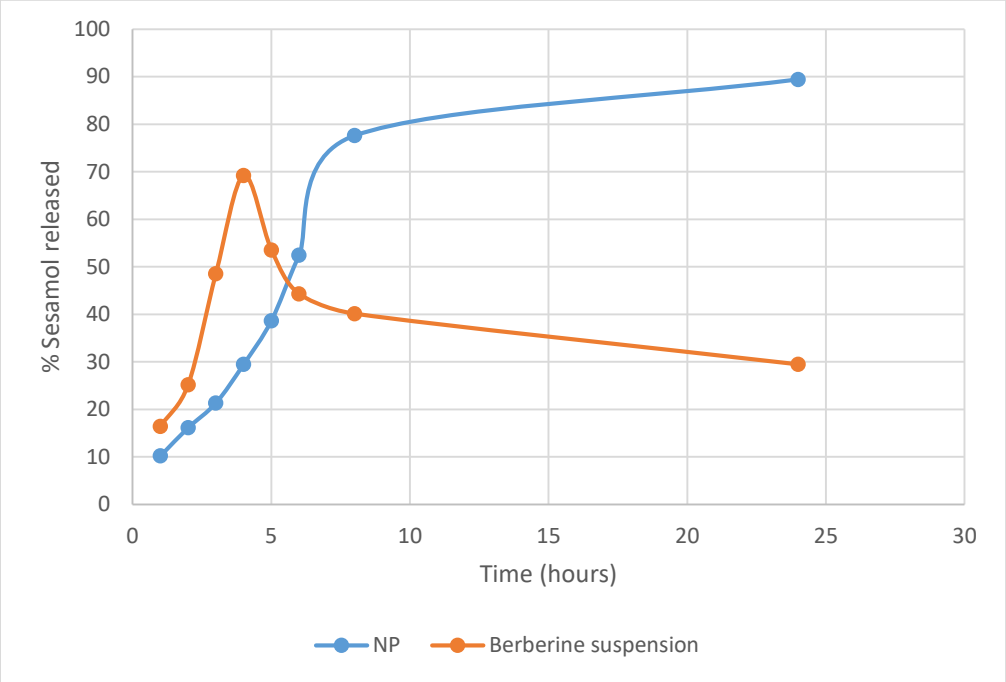


**Figure 3 *In vitro* release of berbereine from solution and zein NP**

After 4 h, 69.18% berberine was released from suspension while only 29.47% berberine was released from zein nanoparticles indicating the ability of nanoparticles to protect the encapsulated berberine. Nevertheless, with time the nanoparticle shell loses its integrity due to formation of aqueous channels leading to swelling of the nanoparticles aiding gradual drug release through such pores (Oh and Flanagan, 2010).

### *In vitro* antioxidant activity of berberine NP

The *in vitro* antioxidant activity of berberine loaded zein nanoparticles was compared with that of berberine solution. The antioxidant action was assessed by DPPH radical scavenging assay method.

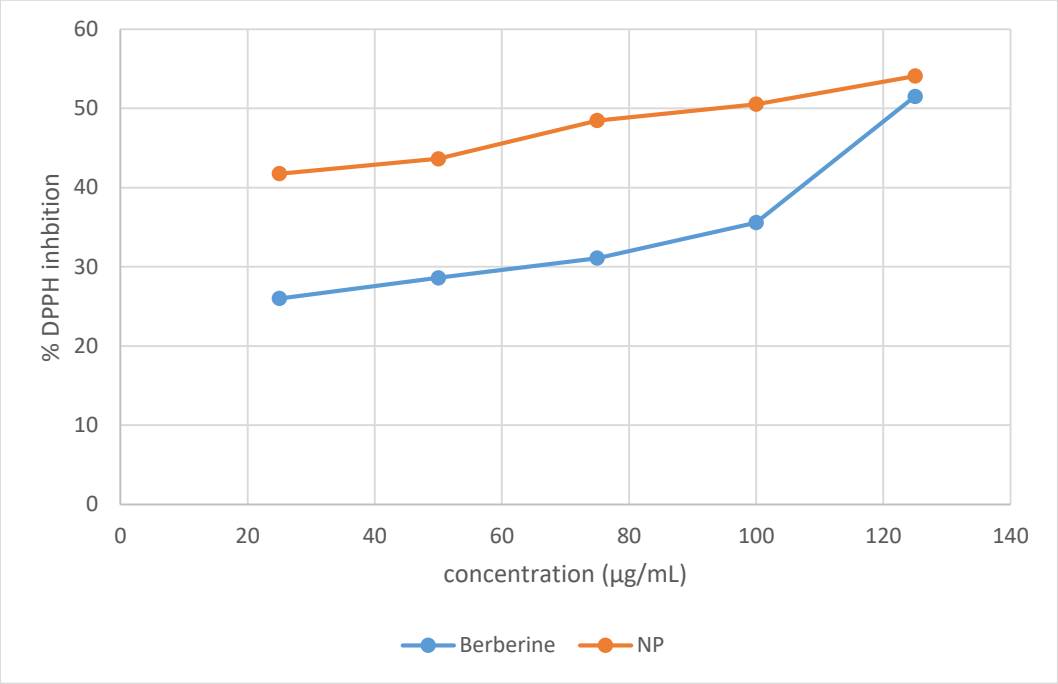


**Figure 4 Antioxidant activity of berberine and berberine loaded nanoparticle**

As it can be visualized from Figure 4, the anti-oxidant activity of the nanoparticle was higher than the berberine solution at all concentrations. This could be due to the stabilization of berberine within the nanoparticle shell (Adel et al., 2023). The $IC_{50}$ value was calculated from the plot and was found to be 122.058 µg/mL and 98.984 µg/mL for berberine and the berberine loaded zein nanoparticle respectively.

## Conclusion

The primary hypothesis for the current work was that incorporation of drugs into shell core nanoparticles prepared by natural substances like zein and pectin could be helpful in stabilizing the drug and improving its bioavailability and efficacy. Berberine is widely regarded as potent antioxidant and is reported to be active against several ailments like cancer and diabetes among others. Berberine was successfully incorporated into zein nanoparticles stabilized by pectin and an improved release and antioxidant profile berberine was found from the nanoparticle formulation. The results led to the conclusion that zein nanoparticles are highly effective in improving the stability, bioavailability and antioxidant action of berberine.

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