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NANOTECHNOLOGICAL APPROACH TO ENHANCE THE STABILITY AND BIOAVAILABILITY OF THE HERBAL DRUG "MURVA"

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ABSTRACT

Murva (*Maerua oblongifolia*) contains numerous bioactive compounds that may provide multiple health benefits, including anti-microbial, anti-fungal, anti-pyretic and anti-diabetic. Most of the therapeutic effects of murva have been attributed due to the presence of triterpenoids and alkaloids, in their composition. Although these compounds have been shown promising therapeutic effects under *in-vitro* conditions, they met with limited efficacy in clinical settings due to various reasons such as poor oral absorption and bioavailability. Different techniques have been proposed to improve the stability and bioavailability of the herbal drugs. Among such strategies, nanoparticulate based drug delivery systems are novel and promising tools. In this study, chitosan nanoparticles containing Murva (CNP1-CNP3) were synthesized by ionic gelation technique, which resulting in particles size smaller than 650nm. The encapsulation efficiency of nanoformulations was over 41.5%. The nanoformulations exhibited slow and sustained *in vitro* release over 99% of drug from the Murva encapsulated chitosan nanoparticles after 24 hours. The synthesized nanoformulations were found to be a promising system for oral sustained administration of murva and also enhance its stability and bioavailability.

Keywords: Nanoparticles, Murva, chitosan, stability, bioavailability.

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INTRODUCTION

Murva is one of the potent phytomolecule, obtained from *Maerua oblongifolia* (Forssk.) A. Rich.) of family Capparaceae, has been traditionally used to cure various diseases[1]. Ethanomedical survey reveals that Murva (*Maerua oblongifolia*) is used to cure various diseases such as fever, stomach ache, skin infections, urinary calculi, diabetes mellitus, epilepsy, pruritis, rigidity in lower limbs, abdominal colic and cough[2]. Most of the therapeutic effects of murva have been attributed due to the presence of triterpenoids and alkaloids, in their composition. Although these compounds have been shown promising therapeutic effects under *in-vitro* conditions, they met with limited efficacy in clinical settings due to various reasons such as poor oral absorption and bioavailability. Different techniques have been proposed to improve the stability and bioavailability of the herbal drugs. Among such strategies, nanoparticulate-based drug delivery systems are novel and promising tools.

Nanoparticulate drug delivery systems may increase drug/bioactive levels by avoiding presystemic hepatic metabolism[3]. Nanoparticulate-based delivery systems may enhance oral absorption of drug/bioactive by increasing the gastric residence time through mucosal adhesion[4] or by increasing cell or tissue entry[5,6] (e.g., Peyer's patches and M cell-mediated uptake). Nanoparticles formulations may reduce the drug exposure to the adverse conditions in the GI tract, thereby minimizing enzymatic and non-enzymatic degradations, and this can result in increased plasma exposure of the bioactive. The encapsulation of

drug/bioactive molecules in nanoparticles can significantly reduce their apparent clearance from plasma, thereby enhancing the apparent drug/bioactive circulation half-life and potential cumulative drug/bioactive delivery to the target tissues[7].

Most of the investigated nanoparticulate-based delivery system for herbal drugs was chitosan nanoparticles. Chitosan, a linear polymer composed of randomly distributed β-(1-4)-linked D- glucosamine and N-acetyl-D-glucosamine, is the only alkaline natural polysaccharide with degradable, biocompatible and film- forming properties[8]. Chitosan possess some unique properties such as controlled release, in situ gelling, mucoadhesion, hydrophilic character, transfection enhancing, permeation enhancing, and efflux pump inhibitory properties, which make it an ideal candidate for drug delivery applications[9-11]. Chitosan possess positive charge and therefore bind strongly to negatively charged surfaces, and this feature is responsible for many of observed biological activities[12]. Chitosan nanoparticles have the ability to prevent oxidation/degradation of herbal drug encapsulated within them in GI tract[13]. Chitosan nanoparticle carrier has been shown to inhibit intestinal P-gp (ABC trans- porter) and enhance nutrient oral absorption[14]. This study was aimed to synthesize Chitosan nanoparticles loaded with Murva to improve the stability and bioavailability by evaluating their entrapment efficiency, particle size and *in-vitro* drug release.

MATERIALS AND METHODS

Materials:

Chitosan, Glacial acetic acid, sodium tripolyphosphate were purchased from Otto Kemi, Mumbai. All other chemicals used were of analytical grade.

Methods:

Preparation of herbal nanoparticles of Murva

Herbal nanoparticles were prepared by ionic gelation method as reported by Calvo. *et. al.*,(1997)[15] with slight modification. The nanoparticles were easily prepared upon the addition of TPP to a chitosan solution containing Murva and mixing by using magnetic stirrer. Encapsulating polymer solution was prepared by dissolving chitosan in glacial acetic acid solution to achieve 1%, 3% and 5% (W/V) of different formulations. Drug solution was prepared separately by dissolving Murva (10 mg) in water. The chitosan and drug solutions were mixed together using magnetic stirrer (25°C, 30 min) and 0.25% (w/v) TPP was added drop-wise while stirring. During the ionic interaction of chitosan with TPP establishes an equilibrium leading to the reduction of the aqueous solubility of chitosan[16]. In this process, it traps the Murva into the core to encapsulate resulting in nanoformulations. The resultant nanoparticles suspension was centrifuged (10,000 rpm, 30 min), the particles were washed thrice with distilled water and freeze dried. While preparing nanoparticles, it is critical to regulate the ratio between chitosan and TPP in order to control the uniform size of the nanoparticles in the reacting mixture. The procedure was repeated for all the three formulations CNP1, CNP2 and CNP3 prepared[17-21].

Table 1: Formula for preparation of Chitosan nanoparticles

S.No	Formulation Code	Murva (mg)	Chitosan (%)	Curcumin : Chitosan ratio	0.25%TPP solution (ml)	0.1% Acetic acid solution (ml)
1	CNP1	10	0.1	1:1	10	100
2	CNP2	10	0.3	1:3	10	100
3	CNP3	10	0.5	1:5	10	100

Evaluation of Entrapment Efficiency

The entrapment efficiency is also known as Association Efficiency. The drug loaded nanoparticles are centrifuged at a high speed of 3500-20000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer at 365nm[22]. The percentage Drug Entrapment Efficiency was calculated as follows:

$$\text{Entrapment efficiency} = \frac{\text{Entrapped drug (mg)}}{\text{Total amount of drug added (mg)}} \text{ X } 100$$

Particle Size analysis

The particle size was analyzed by Zeta sizer Nano ZS (Malvern Instruments, UK). For the analysis, the nanoparticles sample of the desired concentration was flushed through a folded capillary cell (DTS1060) and the measurement was carried out on the second filling; a sufficient sample volume was used to completely cover the electrodes of the cell. The sample was injected slowly and analysis was carried out if there were no visible air bubble inclusions present. After inspection, the cell was placed into the Zetasizer and equilibrated at for 2 min prior to the particle size measurements.

Scanning Electron Microscopy (SEM)

The surface morphology of the chitosan nanoparticles were studied using Scanning electron microscopy (SEM Jeol JSM-6400, JAPAN) operating at 20kv. The samples are mounted on a metal stub with double adhesive type and coated with platinum/palladium alloy under vacuum. The results were given in results and discussion section.

In-vitro Release Studies

Dissolution studies were carried out by using USP dissolution test apparatus. Capsule filled with nanoparticles equivalent to 10 mg of drug was placed in dissolution media in dissolution apparatus. In order to

simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used referred to as sequential pH change method. When performing experiments, the pH 1.2 medium was first used for 2 hours (since the average gastric emptying time is 2 hrs.), then removed and the fresh pH 7.4 phosphate buffer saline was added. After 3 hours (average small intestinal transit time is 3 hrs.), then the medium was removed and colonic fluid pH 6.8 buffer was added for subsequent hours. 900ml of the dissolution medium was used at each time.

Rotation speed was 100 rpm and temperature was maintained at 37±0.5°C. 5 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The samples were withdrawn at specified intervals and analyzed at 365 nm by UV absorption spectroscopy and the cumulative percentage release was calculated over the sampling times.

Accelerated stability study:

The nanoparticles from the selected and optimized batch was studied for stability and kept under the accelerated conditions of temperature and moisture (humidity) for the period of six months. This nanoparticles stability was studied at three different temperature conditions, i.e 4°C, Room Temperature and 45°C for 6 months. Every sample separately weighed and enclosed by aluminium foils and sealed. The samples were kept in specified conditions for a period of six months. The samples were collected at specific time intervals and measured for the amount of drug release using UV-Spectrophotometer at 365nm.

RESULT AND DISCUSSION

Chitosan Nanoparticles: Formulation and Evaluation

Chitosan Nanoparticles were successfully formulated and evaluated. All the formulations were white in colour. The particles obtained were of smooth and free flowing. Chitosan nanoparticles were prepared by ionic gelation technique based on the ionic interaction of a positively charged chitosan solution and negatively charged TPP solution. The charge density of both chitosan and TPP solution has a great effect on the ionic interaction. No visible impurity was seen in the prepared chitosan nanoparticles.

Entrapment Efficiency of Chitosan Nanoparticles

The entrapment efficiency of chitosan nanoparticles increased with increase in polymer concentration. The entrapment efficiencies were found to be minimum and maximum of 41.5±2.6% and 87.3±0.8% respectively. From the drug content and entrapment efficiency results, the formulation CNP5 was considered as an optimum trial. The results were given in the table 2.

Table 2: Drug content and Entrapment efficiency of chitosan nanoparticles

S.No	Formulation Code	Curcumin : Chitosan ratio	Entrapment efficiency (%)
1	CNP1	1:1	41.5±2.6
2	CNP2	1:3	64.7±3.7
3	CNP3	1:5	87.3±0.8

n=3 mean±SD

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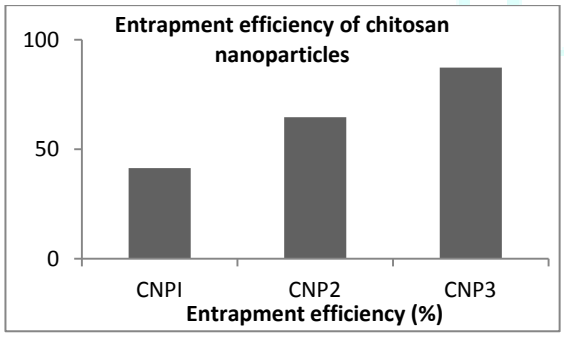

Figure 1: Entrapment efficiency of chitosan nanoparticles

Particle Size of Chitosan Nanoparticles

The particle size of chitosan nanoparticles varied from 360±12nm to 622±42nm. The mean particle size of chitosan nanoparticles was reduced from CNP1 (622±42nm) to CNP3 (360±12nm) with increase in polymer concentration. This may be due to avoidance of aggregation of drug particles. The particle size of the chitosan nanoparticles (CNP1-CNP3) were given in the table 3.

Table 3: Particle Size of chitosan nanoparticles

S.No	Formulation Code	Curcumin : Chitosan ratio	Particle Size (nm)
1	CNP1	1:1	622±42
2	CNP2	1:3	582±42
3	CNP3	1:5	360 ±12

n=3 mean±SD

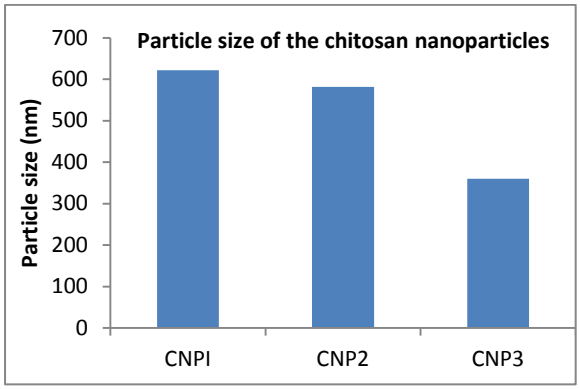

Figure 2: Particle Size of Chitosan Nanoparticles

Scanning Electron Microscopy:

The surface morphology of the selected optimized formulation (CNP3) was determined by scanning electron microscopy (SEM) for characterization of nanoparticles. The result shows that the prepared nanoparticles were spherical, discrete and having a smooth to rough surface.

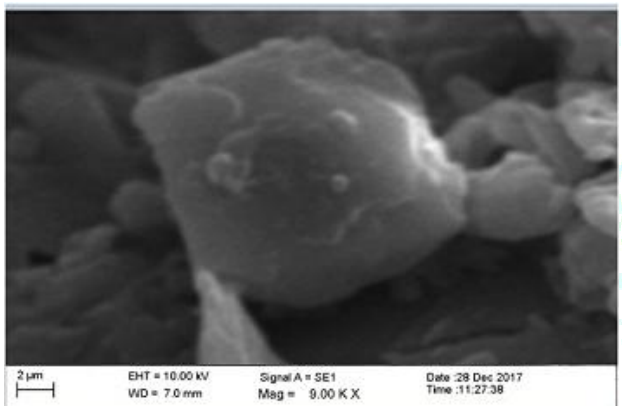

Figure 3: Scanning electron microscopy of CNP3 formulation

***In-Vitro* Release Studies:**

The prepared formulations (CNP1–CNP3) were subjected to *in-vitro* release studies. Absolutely, there was no drug release in simulated gastric fluid (acidic pH 1.2) for initial 2 hours. The drug release was found in simulated intestinal fluid (pH 7.4 phosphate buffer) and in colonic medium (pH 6.8 phosphate buffer).

In-vitro release profiles in intestinal/colonic medium were found to have very good controlled efficacy. During dissolution study it was found that, the drug release depends upon the nature of the polymer matrix as well as the pH of the media. In common increase in polymer concentration produced much more time for release of drug for all formulations. The drug release was decreased and sustained with increase in polymer concentration.

Chitosan nanoparticles prepared with 0.5% chitosan (CNP3) showed controlled and sustained drug release for a period of 24 hr. The percentage cumulative drug release of CNP3 at the end of 24 hr was found to be 99.74±0.26%. The results were given in the table 4.

Table 4: In vitro release of chitosan nanoparticles (CNP1 to CNP3)

Time (hrs)	% Cumulative drug release		
	CNP1	CNP 2	CNP 3
0	0	0	0
1	0	0	0
2	0	0	0
4	45.83±0.85	29.13±0.28	17.17±0.22
6	83.77±0.64	58.88±0.42	35.54±0.09
8	97.36±0.52	85.79±0.31	70.35±0.35
12	98.33±0.36	95.23±0.36	85.39±0.27
16	99.14±0.25	97.65±0.18	91.47±0.46
20	-	99.48±0.25	95.81±0.38
24	-	-	99.74±0.26

n = 3; Mean ± S.D

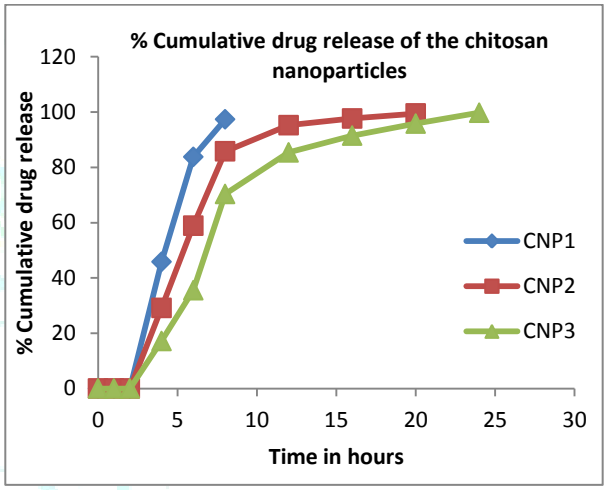

Figure 4: *In-vitro* drug release of the chitosan nanoparticles (CNP1-CNP3)

Stability studies

The prepared nanoparticle formulations were subjected to stability studies as per ICH guidelines. The samples were kept at three different temperature conditions, i.e 4°C, Room Temperature and 45°C for 6 months. Samples were withdrawn at predetermined time intervals of 0, 1, 2, 3 and 6 months and then evaluated for the drug release. The result showed a slight degradation at the elevated temperature (45°C) in comparison with 4°C and room temperature. This suggests that the formulations were stable under normal conditions. However the ideal storage of the nanoparticles formulation at temperatures of 25°C or less may be more suitable for stability purposes. Further, none of the nanoparticles formulations indicated any symptoms of agglomeration or colour change during the period of assessment.

Table 5: Stability study of chitosan nanoparticles (CNP1, CNP2 and CNP3)

Sl. No	Time in months	CNP1 Formulation			CNP2 Formulation			CNP3 Formulation		
		4°C	Room Temp	45°C	4°C	Room Temp	45°C	4°C	Room Temp	45°C
1	0	100	100	100	100	100	100	100	100	100
2	1	97.02	94.99	94.64	98.01	97.89	95.65	99.47	99.05	95.03
3	2	97.67	96.68	92.01	96.55	97.01	94.90	98.11	98.16	95.98
4	3	96.42	97.79	91.44	97.99	98.66	97.10	97.12	97.88	96.12
5	6	94.78	92.04	90.42	96.04	95.67	89.11	96.68	96.21	92.33

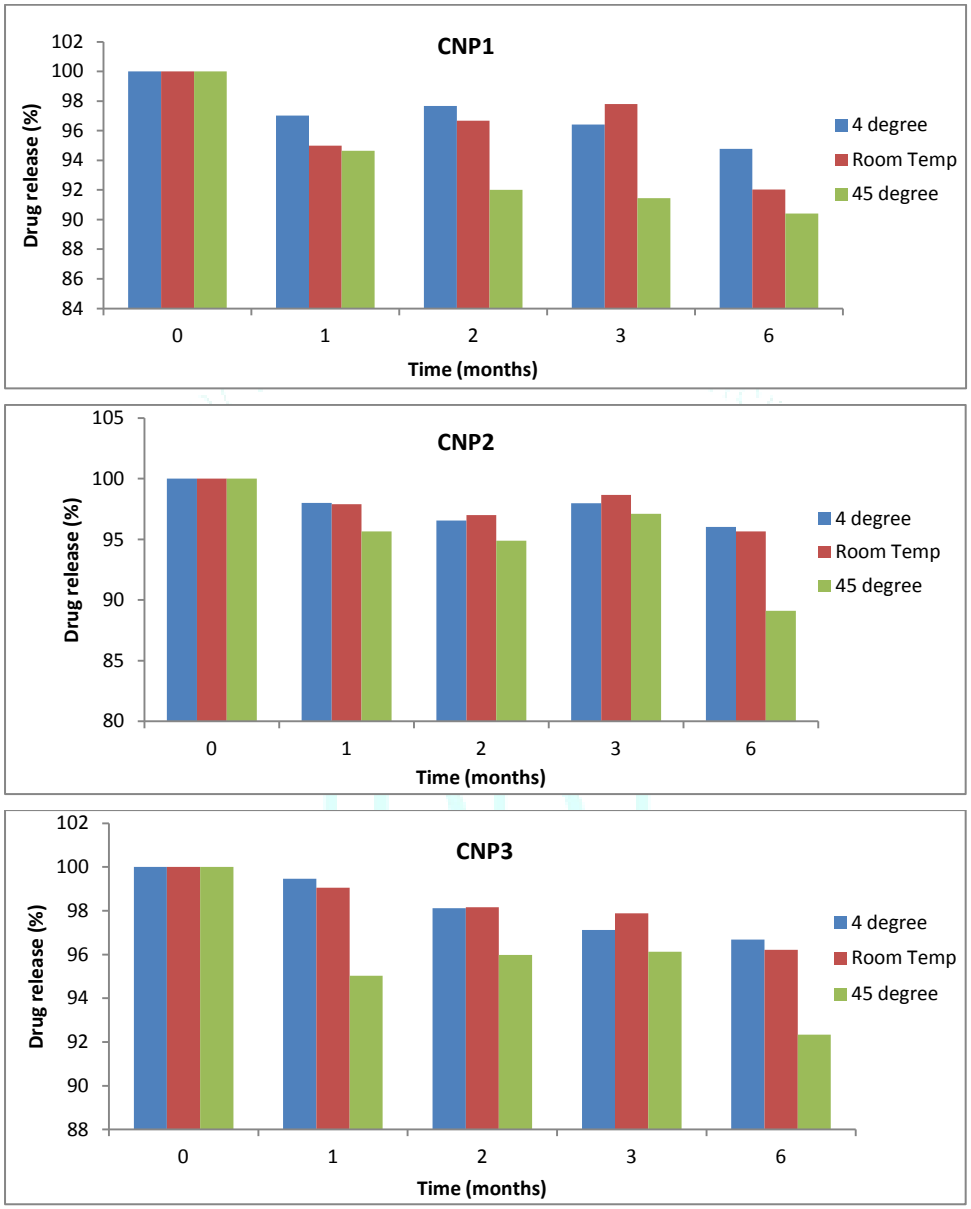

Figure 5: Stability study of chitosan nanoparticles (CNP1, CNP2 and CNP3).

SUMMARY AND CONCLUSION

In the present work, the study was carried out with a view to increase the stability and bioavailability of the herbal drug Murva. Chitosan nanoparticles were successfully prepared by ionic gelation method using TPP as a crosslinking agent by varying the concentration of Chitosan. Chitosan nanoparticles (CNP1-CNP3) showed entrapment efficiency from 41.5-87.3%, particle size ranges from 360-622 nm) and *in-vitro* drug releases were over 99%. The *in-vitro* drug release study showed that the release of the drug from the formulation CNP3 was more sustained when compared with other formulations. The formulations (CNP1, CNP2) showed

quick release of drug. Stability studies confirmed that all the nanoparticles preparations were stable at room temperature and or less may be more suitable for stability purpose. Hence it can be concluded that the newly developed formulation- nanoparticulate drug delivery system of murva with Chitosan is considered to be potential and effective agent to enhance the bioavailability and stability.

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Conflicts of interest: None

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