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FORMULATION AND EVALUATION OF CHITOSAN NANOPARTICLES OF THE HERBAL DRUG "MURVA"

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ABSTRACT

Aim: The aim of this study was to assess the potential of nanoparticles to improve the pharmacokinetics of Murva, with a primary goal of enhancing its bioavailability. **Methods:** Chitosan nanoparticles containing Murva (CNP1-CNP10) formulations were synthesized by ionic gelation technique, using a biodegradable polymer chitosan in varying ratios with tripolyphosphate anions (TPP). The prepared nanoparticles were evaluated for drug content, entrapment efficiency, particle size analysis, surface charge, drug release, surface morphology and release kinetics. **Result:** The chitosan nanoparticles showed drug content, drug entrapment efficiency and particle size in the ranges of 69.7-88.1%, 41.1-87.6% and 360-816nm respectively. The formulation CNP5 showed optimum drug release of 99.74% after 24 hrs. **Conclusion:** The developed Chitosan nanoparticulate system is a promising system for oral sustained administration of Murva and also enhances its bioavailability.

KEYWORDS: Sustained release, Murva, nanoparticles, Chitosan.

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] But its clinical

applications are limited largely due to its poor pharmacokinetics, which results in poor bioavailability.

For the past few decades, investigators are shifting their attention towards the use of drug-loaded nanoparticles for targeted delivery applications.[3,4] Nowadays, due to the technological innovations nontoxic, biocompatible, inexpensive and biodegradable nanoparticles with various colloidal dimensions are being developed to enhance the penetration ability, reduce the frequency of doses, toxicity and to improve the therapeutic efficacy.[5,8] The purpose of this study was to synthesize chitosan nanoparticles (CNP) to improve bioavailability of the Murva by evaluating their drug content, encapsulation efficiency, particle size, surface charge and 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

Following studies were performed to formulate and evaluate Chitosan nanoparticles.

Preformulation Studies

Preformulation testing is an investigation of physical and chemical properties of a drug substance.

Standard calibration curve

Standard graph of the drug was prepared by dissolving different concentrations of standard Murva in pH 7.4 phosphate buffer. The absorbance was measured at 365 nm. Linear relationship was observed with absorption to concentration of drug. The values of absorbance related to concentration were given in results and discussion section.

Fourier Transform Infra Red spectroscopy

IR spectra of Curcumin and other excipients used in the formulation were recorded by using "Thermoscientific." The sample for the IR spectroscopy was prepared by mixing the samples with spectroscopic grade KBr and compressed into transparent pellets, then scanned in the IR range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Preparation of Nanoparticles

The nanoparticles were prepared by ionotropic gelation technique.[9] Briefly, chitosan solution was prepared by dissolving various concentrations of chitosan (0.1 to 1%) in 100ml of acetic acid. 10mg of drug was added to 0.1% TPP solution. This solution was stirred for 1500rpm for 30min on ultrasonicator (vibronics) and TPP solution was added drop wise and kept stirring for 3 hours on homogenizer. Nanoparticles were obtained upon the addition of TPP aqueous solution to chitosan solution. The NP suspension was then centrifuged at 15,000 rpm for 10 min using high- speed centrifuge (Sigma). The formation of nanoparticles results in interaction between the negative groups of TPP and the positively charged amino groups of chitosan.[10] The ratios of drug and polymer are enlisted in the table 1.

Table 1: Formula for preparation of Chitosan nanoparticles.

S.No	Formulation Code	Drug (mg)	Chitosan (%)	Tripolyphosphate solution (ml)	0.1% Acetic acid solution (ml)
1	CNP1	10	0.1	100	100
2	CNP2	10	0.2	100	100
3	CNP3	10	0.3	100	100
4	CNP4	10	0.4	100	100
5	CNP5	10	0.5	100	100
6	CNP6	10	0.6	100	100
7	CNP7	10	0.7	100	100
8	CNP8	10	0.8	100	100
9	CNP9	10	0.9	100	100
10	CNP10	10	1.0	100	100

CNP- Chitosan Nanoparticles containing encapsulated drug (Murva).

Evaluation Parameters

Drug Content

The drug content in each formulation was determined by weighing nanoparticles equivalent to 10mg of drug and dissolving in 100 ml of 7.4 pH phosphate buffer, followed by stirring. The solution was filtered through a 0.45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured spectrophotometrically at 366 nm using 7.4 pH phosphate buffer as blank. The drug content of the prepared nanoparticles was determined by the formula:

$$\text{Drug content} = \frac{\text{Weight of the drug in Nanoparticles (mg)}}{\text{Weight of Nanoparticles (mg)}} \times 100$$

Drug Entrapment Efficiency

The entrapment efficiency is also known as Association Efficiency. The drug loaded nanoparticles are centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer.[11] The percentage Drug Entrapment Efficiency was calculated as follows:

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

Particle Size and Surface Charge

The particle size analysis and zeta-potential measurement were 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^{0}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 366 nm by UV absorption spectroscopy and the cumulative percentage release was calculated over the sampling times.

Release Kinetics Studies

1. Zero – order model: Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation: Qt = Q0 + K0t.

2. First order model: The release of the drug which followed first order kinetics can be expressed by the equation: log Qt = log Q0 + Kt / 2.303.

3. Higuchi model: Higuchi model describes the drug release from several types of matrices initially conceived for planar systems, then extended to different geometrics and porous systems. For Higuchi release kinetics equation is, Q = KH t 0.

4. Koarsmeyar–Peppas model: Koresmeyer derived a simple relationship which describes drug release from a polymeric system. To find out the mechanism of drug release, first 60% drug release data was fitted in Koarsmeyar – Peppas model equation, $(M_t/M) = Km\ t^n$**.**

RESULTS AND DISCUSSIONS

Preformulation studies

Chitosan Nanoparticles were successfully prepared and evaluated. The formulations were coded as CNP1-CNP10. The results and discussions were as follows.

Standard curve of Murva

Standard graph of the drug was prepared by dissolving different concentrations of Murva in phosphate buffer (pH 7.4). The absorbance was measured at 365 nm. Linear relationship was observed with absorption to concentration of drug. The values of absorbance related to concentration were given in table 2.

Table 2: Standard curve of Murva.

S.no	Concentration(µg/ml)	Absorbance(nm)
1	10	0.012
2	20	0.023
3	30	0.033
4	40	0.045
5	50	0.055
6	60	0.066
7	70	0.076
8	80	0.087
9	90	0.098
10	100	0.109

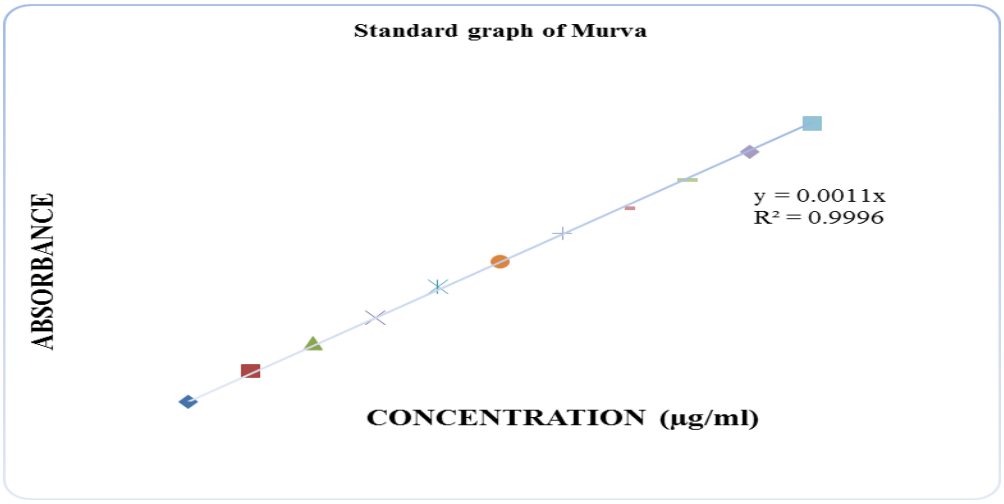

Fig. 1: Standard curve of Murva.

Compatibility study using FTIR

FTIR is extensively used for quantitative analysis and chemical structure determination of compounds to investigate the structure of polymers and to analyze the possible interactions between their functional groups with the drug. The FTIR of the drug Murva, Chitosan polymer and Chitosan nanoparticles are presented in Figure 2-4.

In the FTIR spectra of chitosan, a strong peak in the region of 3500-3300 cm^{-1} was attributed to hydrogen-bonded (O-H) stretching vibration. The peaks of N-H stretching from primary amine and type II amide are overlapped in the same region. The peak for asymmetric stretch of C-O-C was found at around 1380 cm^{-1} and the peak at 1066 cm^{-1} belongs to the C-N stretching vibration of type I amine.

In chitosan nanoparticles the tip of the peak of 3450 cm^{-1} has a shift to 3272 cm^{-1} and becomes wider with increased relative intensity indicating an enhancement of hydrogen

bonding. In nanoparticles the peaks for N-H bending vibration of amine I at 1600 cm^{-1} and the amide II carbonyl stretch at 1650cm^{-1} shifted to 1450 cm^{-1} and 1621 cm^{-1} respectively. The characteristic peak of C-N (1000- 1400 cm^{-1}) is present in FTIR of Chitosan polymer along with other peaks. But in chitosan nanoparticles the peak was shifted due to the wagging of NH_2 bond. The ionic interaction with the phosphate group of TPP indicated the conversion of chitosan polymer in the nano form, that forms a cross link with TPP. The strong and sharp peak of phosphate at 1102cm^{-1} in chitosan nanoparticles confirmed the involvement of TPP while making the nanoparticles. These changes in FTIR Spectrum of Chitosan nanoparticles were observed from the Chitosan FTIR spectra due to the entrapment of drug that is clearly observed.

Hence from the FTIR study, the characteristics peaks are present and it was found that there was no interaction took place between the drug and other ingredients used in the formulation. So it was concluded that the drug was found to be compatible with other excipients used in the formulation.

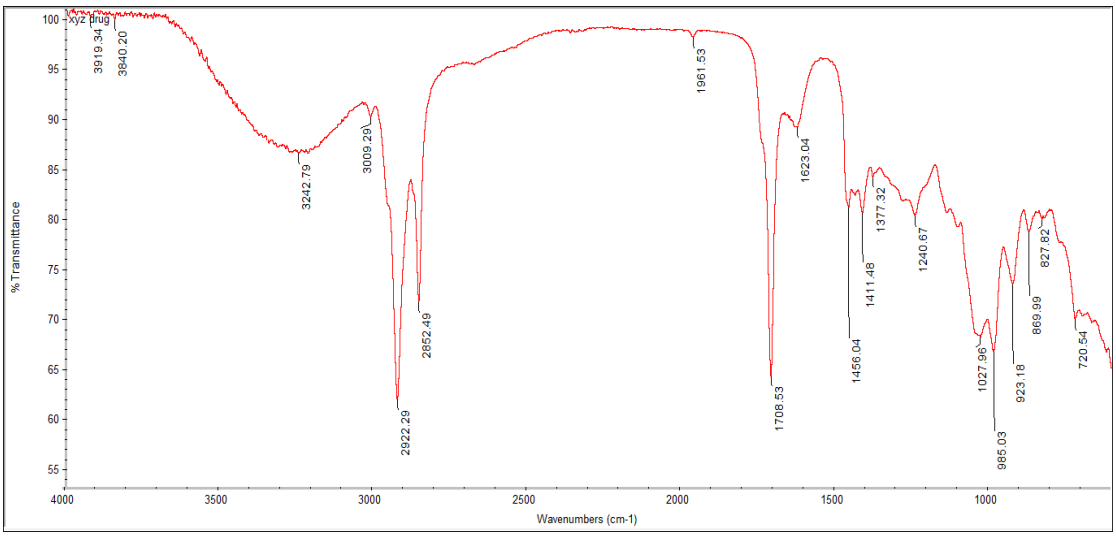

Fig. 2: FTIR Spectrum of drug Murva.

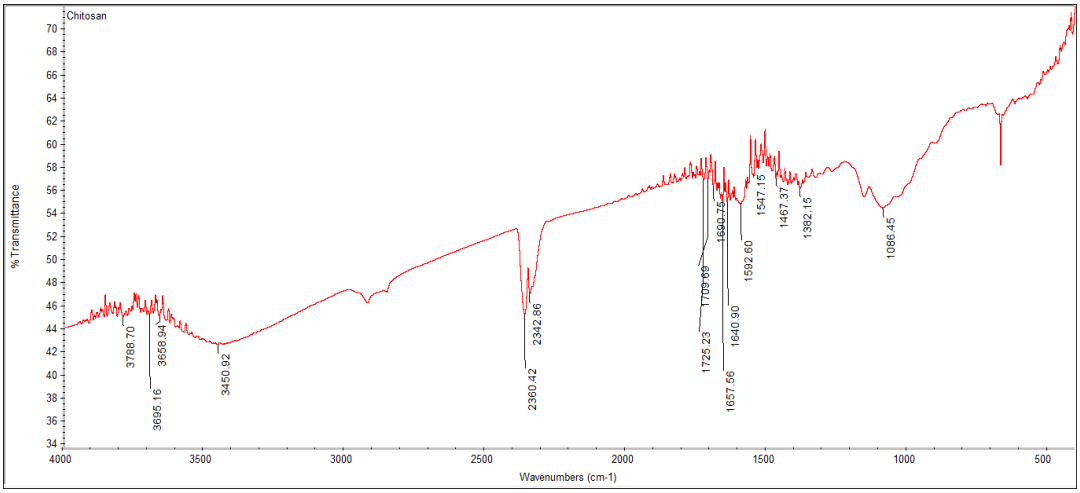

Fig. 3: FTIR Spectrum of Chitosan.

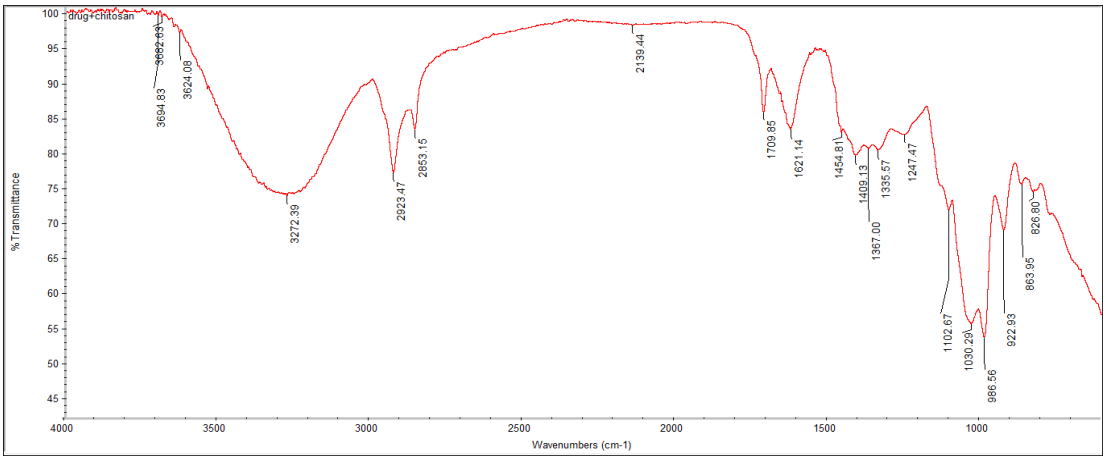

Fig. 4: FTIR Spectrum of Chitosan nanoparticles.

Chitosan Nanoparticles – Formulation and Evaluation

Chitosan Nanoparticles were successfully prepared 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. The chitosan nanoparticles were prepared 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 the prepared chitosan nanoparticles.

Drug Content and Entrapment Efficiency of Chitosan Nanoparticles

The drug content of the chitosan nanoparticles varied from 69.7±7.2% to 88.1±1.2%. The drug content was decreased with increase in chitosan concentration. This may be due to loss of drug during manufacturing stage or increase in entrapment efficiency, so that the drug is not available for estimation.

The entrapment efficiency of chitosan nanoparticles increased with increase in polymer concentration upto 0.5% chitosan (CNP5). After that, there was no significant increase in entrapment efficiency. This may due to unavailability of drug for entrapment. The entrapment efficiencies were found to be minimum and maximum of 41.5±2.6% and 87.6±1.2% respectively. But the maximum entrapment efficiency was not considered as optimum. The optimum percentage efficiency was based on the drug content and polymer usage. The optimum entrapment efficiency was found to be 87.3±0.8%. From the drug content and entrapment efficiency results chitosan nanoparticles CNP5 was considered as optimum trial. The results were given in the table 3.

Table 3: Drug content and Entrapment efficiency of chitosan nanoparticles (CNP1-CNP10).

S.No	Formulation Code	Drug content (%)	Entrapment efficiency (%)
1	CNP1	88.1±1.2	41.5±2.6
2	CNP2	87.9±2.4	57.5±2.4
3	CNP3	87.5±2.6	64.7±3.7
4	CNP4	86.9±3.2	75.6±0.8
5	CNP5	86.3±0.6	87.3±0.8
6	CNP6	84.5±2.6	86.3±3.6
7	CNP7	80.6±3.2	87.4±2.9
8	CNP8	78.5±6.8	87.6±1.2
9	CNP9	76.1±6.8	86.9±1.4
10	CNP10	69.7±7.2	85.3±3.6

n=3 mean±SD

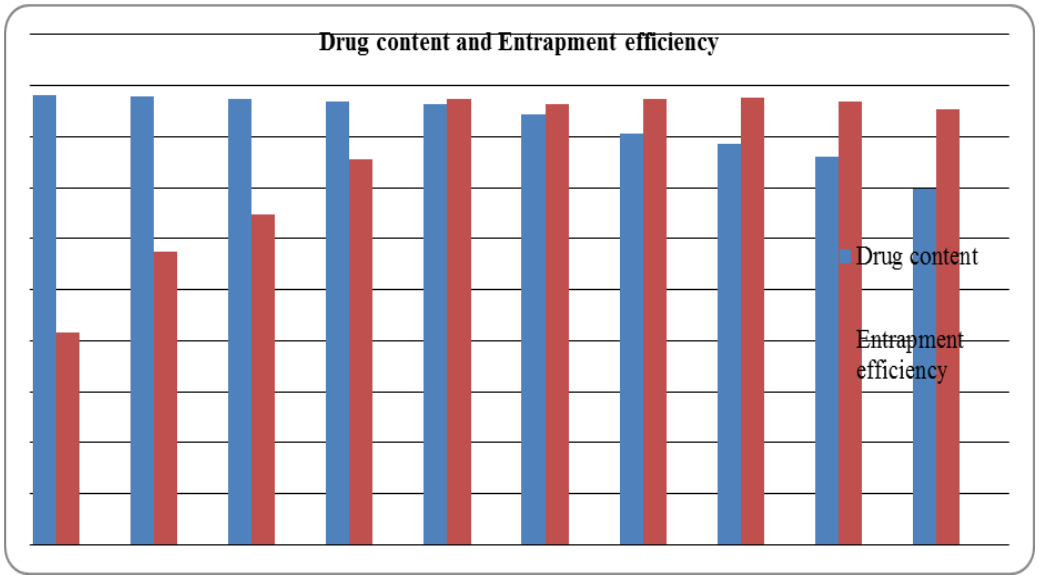

Fig. 5: Drug content and Entrapment efficiency of chitosan nanoparticles.

Particle Size of Chitosan Nanoparticles

The particle size of chitosan nanoparticles varied from 360±12nm to 816±62nm. The mean particle size of chitosan nanoparticles was reduced from CNP1 (622±42nm) to CNP5 (360±12nm) with increase in polymer concentration. This may be due to avoidance of aggregation of drug particles. But the particle size was increased from CNP5 (360±12nm) to CNP10 (816±62nm) due to increase in polymer concentration. The results were given in the table 4.

Table 4: Particle Size of chitosan nanoparticles (CNP1-CNP10).

S.No	Formulation Code	Particle Size (nm)
1	CNP1	622±42
2	CNP2	604±36
3	CNP3	582±42
4	CNP4	460±42
5	CNP5	360 ±12
6	CNP6	480±78
7	CNP7	520±76
8	CNP8	625±55
9	CNP9	715±82
10	CNP10	816±62

n=3 mean±SD

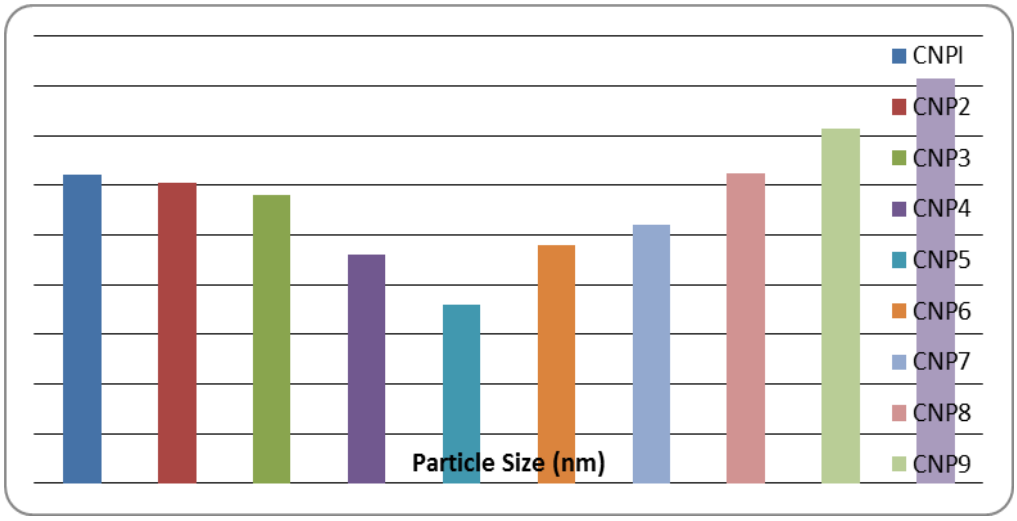

Fig. 6: Particle Size of Chitosan Nanoparticles.

Zeta potential of Chitosan Nanoparticles

The zeta potential values of chitosan nanoparticles were in positive and increased from 11.2±1.2mV to 18.7±0.4mV. The positive value was due to the polar nature (NH_3 group) of chitosan. The CNP5 trial held a value of 18.3±0.2mV. There was significant increase in zeta

potential value from CNP1 to CNP5, but from CNP5 to CNP10 there was no significant increase in zeta potential value. Hence from these studies, formulation CNP5 (18.3±0.2mV) was considered as optimum trial. The results were shown in the table 5.

Table 5: Zeta potential of chitosan nanoparticles (CNP1-CNP10).

S.No	Formulation Code	Zeta potential(mV)
1	CNP1	11.2±1.2
2	CNP2	12.2±1.3
3	CNP3	13.4±1.4
4	CNP4	14.3±0.6
5	CNP5	18.3±0.2
6	CNP6	18.4±0.4
7	CNP7	18.1±0.5
8	CNP8	18.4±0.2
9	CNP9	18.6±0.2
10	CNP10	18.7±0.4

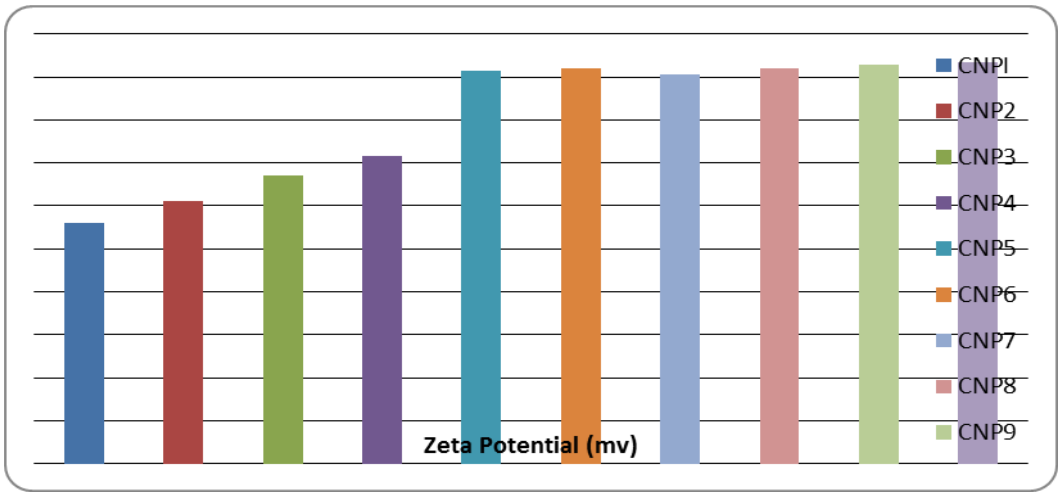

Fig. 7: Zeta Potential of Chitosan Nanoparticles.

***In-Vitro* Release Studies**

The prepared formulations (CNP1 - CNP10) 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.

More concentration of polymer (i.e ≥0.6% Chitosan CNP6 to CNP10) formed slow release of drug for more than 24 hr. Less concentration of polymer (i.e ≤ 0.5% Chitosan CNP1 to CNP4) formed quick release of drug within short period. Hence the formulations (CNP1-CNP4, CNP6-CNP10) were considered to be not satisfactory for controlled delivery of drug either by quick release or over retarding. Chitosan nanoparticles prepared with 0.5% chitosan (CNP5) showed controlled and sustained drug release for a period of 24 hr. The percentage cumulative drug release of CNP5 at the end of 24 hr was found to be 99.74±0.26%. The results were given in the table 7.

Table 7: In vitro release of chitosan nanoparticles (CNP1 to CNP10).

Time (hrs)	% Cumulative drug release									
	CNP 1	CNP 2	CNP 3	CNP 4	CNP 5	CNP 6	CNP 7	CNP 8	CNP 9	CNP 10
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
4	45.83 ±0.85	33.26 ±0.17	29.13 ±0.28	28.58 ±0.36	17.17 ±0.22	16.87 ±0.42	15.28 ±0.18	13.32 ± 0.22	12.46 ±0.16	11.52 ±0.31
6	83.77 ±0.64	68.69 ±0.34	58.88 ±0.42	57.82 ±0.24	35.54 ±0.09	34.35 ±0.47	32.42 ±0.35	29.54 ±0.31	27.52 ±0.47	25.28 ±0.39
8	97.36 ±0.52	90.24 ±0.49	85.79 ±0.31	83.37 ±0.32	70.35 ±0.35	67.36 ±0.57	65.64 ±0.28	63.47 ±0.81	61.74 ±0.61	59.79 ±0.83
12	98.33 ±0.36	97.27 ±0.67	95.23 ±0.36	95.58 ±0.45	85.39 ±0.27	76.43 ±0.38	74.48 ±0.62	69.72 ±0.34	66.84± 0.28	63.28 ±0.08
16	99.14 ±0.25	99.22 ±0.71	99.48 ±0.25	98.15 ±0.58	91.47 ±0.46	84.17 ±0.24	79.55 ±0.51	76.38 ±0.26	73.45 ±0.75	70.57 ±0.53
20	99.25 ±0.09	99.26 ±0.54	99.49 ±0.18	99.24 ±0.12	95.81 ±0.38	91.13 ±0.63	87.47 ±0.36	81.52 ±0.27	77.72 ±0.64	75.48 ±0.36
24	99.35 ±0.34	99.44 ±0.35	99.54 ±0.07	99.66 ±0.14	99.74 ±0.26	96.58 ±0.29	90.28 ±0.23	85.57 ±0.22	80.19 ±0.16	77.13 ±0.51

n=3 mean±SD

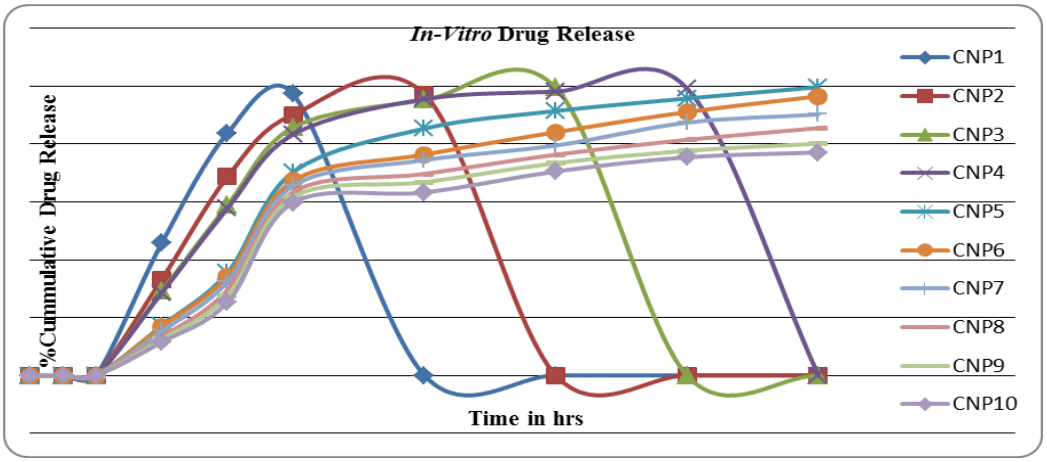

Fig. 8: *In-vitro* release of chitosan nanoparticles (CNP1 to CNP10).

Scanning Electron Microscopy

The surface morphology of the selected optimized formulation (CNP5) 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.

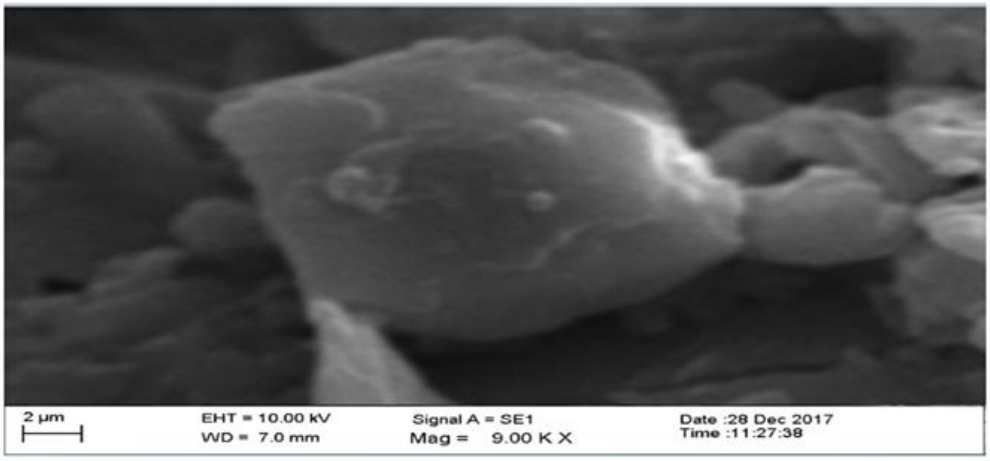

Fig. 9: Scanning electron microscopy of CNP5 formulation.

Release kinetics

To know the mechanism of drug release from various preparations the data were treated according to zero order, first order, Higuchi and Koarsmeyar equation. The release rate kinetic data for all zero order and Koarsmeyar equation were shown in Fig 9,10. The value fitted to zero order plot and its regression value was 0.9465, as its value is close to 1, it was conformed that it followed zero order release. The mechanism of drug release was further conformed by Koarsmeyar and peppas plot. According to this 0.5 is Fickian diffusion, 0.5 < n < 1 is anomalous transport or Non-Fickian transport, 1 is Case II transport, n > 1 is Super case II transport. The value of 'n' was found to be 2.3182 and hence it suggests Super case II transport. The results were presented in the table 8.

Table 8: Release kinetic study of CNP5 formulation.

Formulation code	Zero order R^2	First order R^2	Higuchi R^2	Koarsmeyar-peppas	
				R^2	N
CNP5	0.9465	0.867	0.8613	0.9189	2.3182

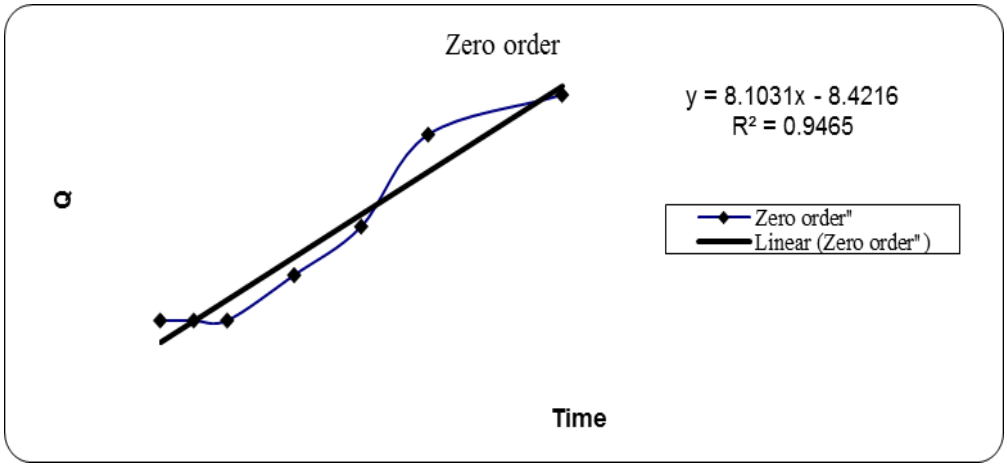

Fig. 10: Zero order release for CNP5 formulation.

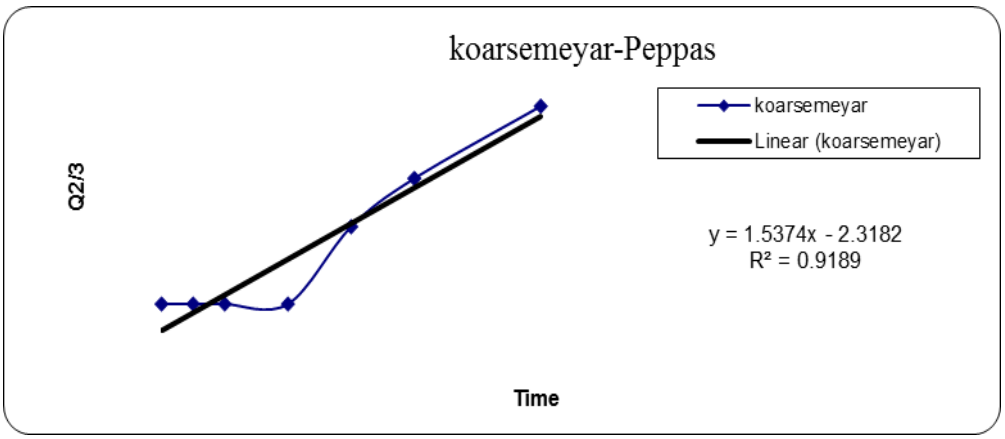

Fig. 11: Koarsmeyer peppas release for CNP5 formulation.

SUMMARY AND CONCLUSION

Chitosan nanoparticles were successfully formulated by ionic gelation method and evaluated for percentage drug content, entrapment efficiency, particle size analysis, surface charge, SEM analysis, *in-vitro* release studies and release kinetics.

From the FTIR spectra it is concluded that, there was no interactions between the drug and excipients since the characteristic peaks of the chitosan remained in the formulations also. The percentage drug content ranged from 69.7-88.1%. The entrapment efficiency was ranged from 41.1-87.6%. The size of the nanoparticles was ranged from 360-816nm. The *in-vitro* drug release was 99.74% for CNP5 formulation at the end of 24 hrs, hence chitosan nanoparticles can provide prolonged release of drug. It is inferred from the release kinetics that the mechanism of release was Super case II transport, since the value of n > 1. From the SEM analysis, the nanoparticles were found to be spherical, discrete and having a smooth to rough surface. Hence, Chitosan nanoparticles formulations offer a promising avenue to

enhance the bioavailabity of the herbal drug murva. It can maintain, sustain and control the drug activity and also minimizes the frequency of drug administration with better patient compliance.

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