INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

FORMULATION AND EVALUATION OF CHITOSAN BASED DICLOFENAC SODIUM NANOPARTICLE FOR TREATING OSTEOARTHRITIC INFLAMMATION BY INTRA-ARTICULAR ROUTE

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ABSTRACT

Osteoarthritis (OA) is a chronic degenerative joint disorder characterized by destruction of the articular cartilage, subchondral bone alterations and synovitis. This synovitis is due to decreased synovial fluid volume at joints, this leads to friction develops between joints and inflammation also occurred. To reduce this inflammation NSAIDs are drug of choice. Since the half-life of Diclofenac Sodium is 3 times greater in synovial fluid than compared to plasma and having good anti inflammation activity. Diclofenac Sodium is having some disadvantages, this drug causes gastric hemorrhage, gastric irritation and the dosing frequency is twice or thrice a day. To avoid these complications, reduce dosing frequency and for controlled release and localized action of drug, nanoparticles in suspension form is suitable for therapeutic efficacy. In this present work, nanoparticles were prepared by ionic gelation method using Chitosan as polymer. This cationic polymer forms gel like structure when interact with negatively charged hyaluronic acid present in synovial fluid. This formed gel increases the viscosity and reduce friction between synovial joints with increase in flexibility of moment. Nanoparticles were prepared by using different concentrations of polymer. The best formulation was optimized and evaluated by using different parameters. F1 formulation showed 92.33±3.50% entrapment efficiency, 64.90±2.29% loading efficiency and 80.6±3.50% drug release for 14 days. *In vivo* study was conducted in osteoarthritis induced (carrageenan and kaolin) rats.

Key words: Diclofenac Sodium, Chitosan, Osteoarthritis, Synovial fluid Nanoparticles, Intraarticular route,

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INTRODUCTION

Now a days modern drug therapy is designed to optimize the pharmacological action of drugs coupled with the reduction of their toxic side effects *in vivo*. macromolecular material which can be of synthetic or natural origin". Depending on the process used for their preparation, two different types of nanoparticles can be obtained i).Nanospheres ii). Nanocapsules. Nanospheres have a matrix type structure in which a drug is dispersed, whereas nanocapsules exhibit a membrane-wall structure with an oily core containing the drug. Because these systems have very high surface areas, drugs may also be adsorbed on their surface (1-3). Diclofenac Sodium is Sodium 2-[(2,6-dichlorophenyl)-amino]phenylacetate use d as anti-inflammatory drug. iclofenac Sodium is one of the NSAIDs that

accumulates in synovial fluid with half-life in this compartment is triple that of the plasma.

The main aim of the present work is to prepare Chitosan based Diclofenac Sodium nanoparticles and optimizes the formulation. To perform physical and chemical evaluation studies like entrapment efficiency, loading efficiency, *in vitro* drug release studies and *in vivo* studies for the optimized formulation in the form of injectable dosage form administered by intra-articular route.

MATERIALS AND METHODS

MATERIALS

Diclofenac Sodium (APEX lab, Chennai), Chitosan (Otto Chemicals and Bio reagents), Sodium tri polyphosphate (STPP) (LOBA chemicals,Pvt. Ltd), Tween 80 (MERCK (Inia) Limited), Ketamine HCl 500mg Inj (Nean Laboratories LTD), Xylazine Inj 10ml (Indian Immunologicals LTD) λ-Carrageenan (Sd fine Chem Limited) and Methanol (Merck Specialities Pvt Ltd).

METHODS

Preparation of Nanoparticles

Nanoparticles were prepared by ionic gelation method. In this method mainly 4 steps are involved **Step 1:** Chitosan was dissolved in 1 per cent glacial acetic acid and stirred under magnetic stirrer until a clear solution was formed. A concentration of 0.5 per cent w/v was prepared for the formulation. **Step 2:** Diclofenac Sodium was dissolved in distilled water and mixed with Chitosan solution drop wise using syringe in the rate of 1 ml/min under constant stirring rate of 1500 rpm. Stirring was continued to form an uniform solution. **Step 3:** 10 ml of 0.1 per cent w/v Sodium tripolyphosphate was added to the above solution and stirring was continued for 1 hr.**Step 4:** After stirring the solution was centrifuged at 16,000 rpm for 30 min. the sediment formed were collected by repeated washing with distilled water. Finally the particles were subjected to freeze drying for 24 hrs and stored for further studies. Formulations were made with different concentrations of Chitosan solutions like 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% w/v as given in the table 8 and stored for further studies (4).

Characterization of Nanoparticles (5-7)

The prepared nanoparticles were subjected to various evaluation studies. **Determination of zeta potential**

The zeta potential of the drug loaded Chitosan nanoparticles were measured by using Zetasizer. (Malvern Instrument LTD, Model: Zetasizer 3000).

Measurement of particle size and morphology

The particle size and surface morphology of the nanoparticles was measured by using Scanning Electron Microscopy (SEM).

***In vitro* drug release studies**

Construction of Calibration Curve of Diclofenac Sodium in Phosphate buffer pH 7.4

Accurately weighed 100 mg of Diclofenac Sodium was transferred into 100 ml volumetric flask and the volume was made upto 100 ml with Phosphate buffer pH 7.4. This solution was labeled as primary stock solution. From this stock solution 1 ml was taken and transferred into 100 ml volumetric flask and the volume was made upto 100 ml with Phosphate buffer pH 7.4. From this secondary stock solution, solutions of various concentrations of 2, 4, 6, 8, 10 µg/ml were prepared. Absorbances of these concentrations were observed at 277 nm by using UV-Visible spectrophotometer.

Procedure

100 mg of nanoparticles were dispersed in 100 ml of Phosphate buffer saline pH 7.4 in a sealed jacketed beaker at 37 ± 0.5^0C. The suspension was stirred continuously at a constant rate using magnetic bead and stirrer aliquots of 1 ml were taken at various time intervals upto 14 days. The drug content in the dissolution medium was determined using the sedimenting the nanoparticles by centrifugation at 16,000 rpm for 15 min then the supernatant was collected and analyzed by using UV-Visible spectrophotometer at 277 nm. The sediment was replaced with 1ml of buffer solution to maintain the sink condition.

The % drug release of Diclofenac Sodium in the nanoparticles was calculated (8-10)

***In Vivo* Studies**

Adult male albino rats of wristar strain weighing about (200-250 g) were purchased from Raghavendra enterprises, Bangalore. The animals were divided into three groups(3x4=12), housed in an air conditioned

room under standard conditions (25 ± 2^0C) and 12 hrs light, 12 hrs dark cycle. Rats had free access to standard diet and purified drinking water. All experiments described in the present study were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy (NO: 1016/a/06/CPCSEA/012/2012).

Diclofenac Sodium nanoparticles were made for suspension by using saline solution before administration. Healthy adult male wistar rats were randomly divided into 3 groups (n=4) as Normal, Control, Test (Table-1).

Table-1 Grouping of animals for *In vivo* studies

S.No.	GROUP	TREATMENT	PURPOSE
I	Normal	Saline solution	Serves as normal
II	Control	Kaolin(4% w/v) + Carrageenan(2% w/v)	To induce OA
III	Test	Drug loaded nanoparticles	To study the effect of formulation on osteoarthritis

Induction of Osteoarthritis

The rats (Group II & III) were anesthetized with ketamine (50 mg/kg bw) and xylazine (12 mg/kg bw) inj. through intraperitoneal route. The skin over the knee was disinfected with povidone iodide, and arthritis was then induced with a single intra-articular injection of 75 µl of a mixture of 2% λ-carrageenan and 4% kaolin in saline. The contralateral knees were injected with saline. After recovering from the anesthesia, the animals were returned to their cages. After 48 hrs group II and group III rats were exposed to X-Ray radiation for identification of osteoarthritis (11, 12).

Injection of nanoparticle suspension in intra-articular space

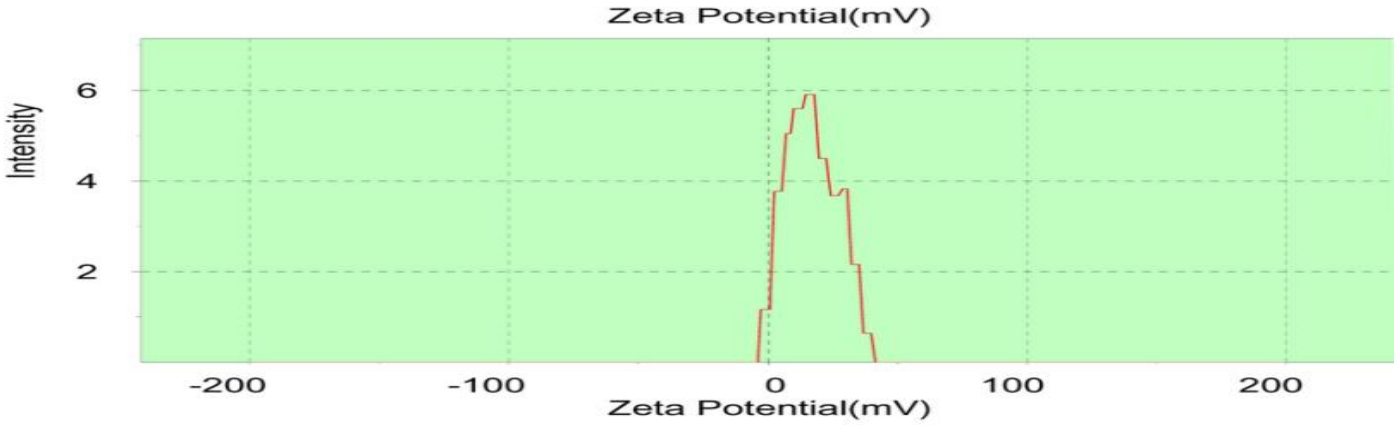
After confirmation of osteoarthritis was developed, group III rats were anesthetized. The prepared nanoparticle suspension was given in intra-articular route which was previously prepared with buffer pH 7.4. All group III rats were injected with dose of 30 µl/kg body weight. After recovering from the anesthesia, the animals were returned to their cages. These were examined for gel formation in the knee joint area under X-Ray radiation (13, 14).

RESULTS AND DISCUSSION

Preformulation studies

In this study the characteristic peaks observed were similar to that of pure drug and polymer. Shifting of NH band from 2920.58 – 3586 was observed in physical mixture due to increase in hydrogen bonding. Based on the information from the drug excipient compatibility studies, there was no incompatible found between drug and polymer.

Zeta Potential

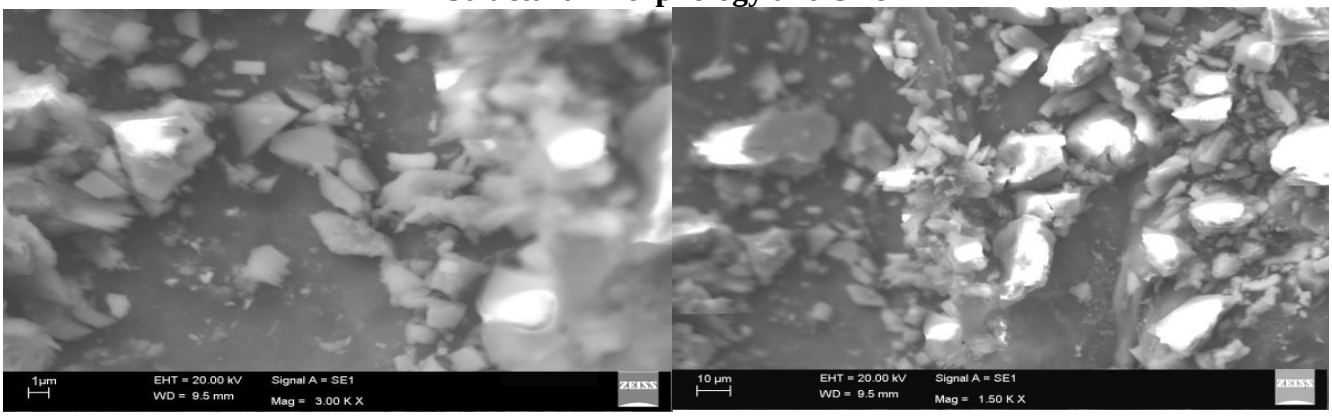


ISSN: 2395-0536

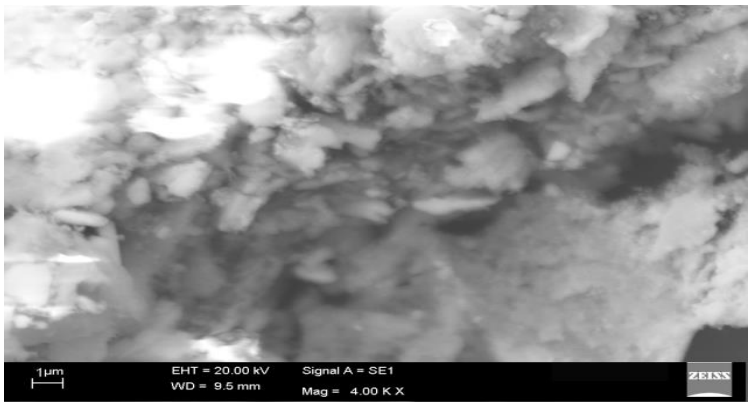
Figure-1 Zeta potential of drug loaded Chitosan Nanoparticles

The particle surface charge distribution ranged from 20.33 mV (Chitosan) to 23.17 mV (Diclofenac Sodium). The positive surface charge is indicative that Chitosan is organized at the surface of the nanoparticle (Fig-1).

Structural Morphology and Size



15a **15b**



15c

Figure-2 SEM images of nanoparticles

Scanning electron microscope (SEM) (Fig-2) revealed that the nanoparticles were in nano size range (600-1000 nm) with the magnification of 3.00kx and at higher magnification of 4.00kx particles were observed in irregular shape. Stirring speed and time depends on particle size and shape of nanoparticles in range. Thickness of the magnetic bead or shaft may also affect the morphology of the nanoparticles. It has been found that particle size affects the drug release. Smaller particles offer larger surface area. As a result, most of the drug loaded onto them will be exposed to the particle surface leading to fast drug release. As a drawback, smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion. Hence, there is a compromise between a small size and maximum stability of nanoparticles.

Entrapment Efficiency and Loading Efficiency

Entrapment efficiency and Loading efficiency results were shown in the table -2 and 3. From this data, it was confirmed that formulation F1 has more entrapment efficiency (92.33 ± 3.05) and more Loading efficiency (64.90 ± 2.29). The encapsulation efficiency of Chitosan nanoparticles has been shown to be inversely proportional to Chitosan concentration and viscosity and drug concentration. To increase the loading efficiency of hydrophobic drugs and controls the release of drug from various Chitosan nanoparticle preparations.

Table-2% Entrapment efficiency (At different stirring speeds)

S. No	Stirring Speed (RPM)	% Entrapment efficiency					
		F1	F2	F3	F4	F5	F6
1	1500	93.46±1.56	85.67±0.65	94.67±2.44	89.33±2.34	84.31±0.77	83.45±1.09
2	2000	89.54±1.39	79.65±0.87	86.29±0.67	86.16±1.06	83.38±1.87	80.12±0.54
3	2500	84.31±1.87	71.89±0.77	91.04±2.32	88.90±0.93	81.56±1.90	79.59±2.89
4	3000	81.45±3.05	68.24±1.78	85.52±1.76	83.93±3.90	76.15±4.01	77.46±3.78

Table-3 % Loading efficiency (At different stirring speeds)

S. No	Stirring Speed (RPM)	% Loading efficiency					
		F1	F2	F3	F4	F5	F6
1	1500	62.3±0.45	41.92±2.99	36.34±1.87	28.45±0.92	23.50±2.78	19.83±1.50
2	2000	59.68±1.33	38.97±3.33	35.00±0.93	27.44±2.43	23.24±2.09	19.03±2.78
3	2500	56.20±1.89	35.17±1.87	34.10±1.39	28.31±1.87	22.73±3.05	18.91±0.84
4	3000	54.29±2.43	33.39±2.54	33.50±2.34	26.73±3.05	21.22±3.11	18.40±1.54

***In vitro* release studies:**Incorporation of Diclofenac Sodium in the nanoparticle formulation had a significant effect on the rate of drug release from nanoparticles. Various concentrations of Chitosan formulations of nanoparticles like F1(0.5%), F2(1.0%), F3(1.5%), F4(2.0%), F5(2.5%), F6(3.0%) had significant effect on rate of release of Diclofenac Sodium from the nanoparticles. According to the results obtained, it was noted that, the percentage release at the end of 14^{th} day was 80.6±3.50, 70.11±4.32, 51.12±2.98, 41.9±1.90, 33.4±2.34, 29.17±6.98 respectively. This showed that the order of rate of release of various formulations was found to be F1>F2>F3>F4>F5>F6 formulations. It can be concluded that F1 formulation had a highest release than the other formulations.

Release Kinetic studies: Release kinetics of drug shows initially first order effect. Gradually, the effect limits to zero order release. We can conclude that the formulation has both first order and zero order release effect to maintain the therapeutic efficacy and decreased toxic effect (Table-4).

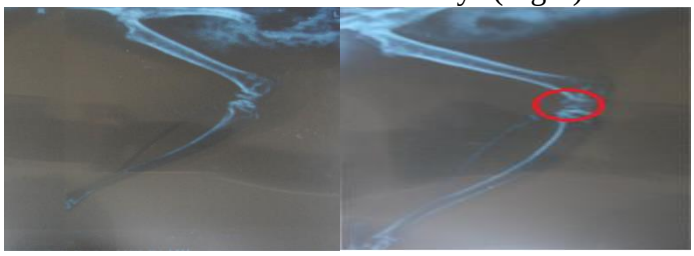
Table-4 Release kinetics data

S. No	Sampling intervals (Days)	Cumulative % Drug release	Log Cumulative % Drug remaining
1	0	0	2.0000
2	1	10.8	1.9563
3	2	17.9	1.9143
4	3	21.3	1.8959
5	4	27.5	1.8603
6	5	34.6	1.8155
7	6	41.7	1.7656
8	7	48.6	1.7109
9	8	55.6	1.6473
10	9	62.1	1.5786
11	10	69.5	1.4842
12	11	77.2	1.3579
13	12	78.9	1.3242

14	13	79.6	1.3096

***IN VIVO* studies**

From the X-Ray pictures of knee joint it was observed that Group II rats (OA induced rats) develop some cartilage destruction and swelling at knee region than compared to normal after 48 hr of induction. Group III rats showed that gel like appearance at joint region after injection of nanoparticles, this gel like appearance was gradually decreased and rats also shown free movement within 14 days (Fig-3).



Normal knee **OA induced knee**

After administration of nanopartcles **7th day** **14th day**

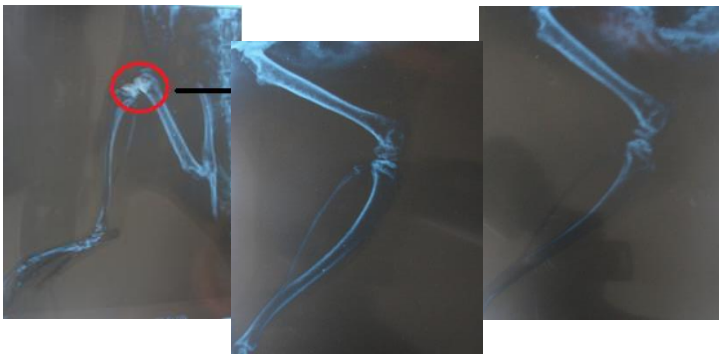


Figure-3 X-Ray images of knee joint

CONCLUSION

Intra-articular drug delivery systems for OA or RA represent a valuable means to administer drugs directly in the joint cavity, while circumventing systemic toxicity and reducing leakage or exposure of other tissues and organs. Due to its encapsulation in a delivery system, the active substance is gradually released, and it can act locally to diminish joint inflammation, in the case of nonsteroidal or steroidal drugs, to remove the synovial lining. Clinical efficiency is required for any novel drug delivery system. Chitosan-TPP Nanoparticles have a novel controlled targeted drug delivery which offer several potential benefits. The aim of the present study was to develop Diclofenac sodium loaded Chitosan-TPP Nanoparticles. Drug/polymer ratio in the nanoparticles, stirring speed influence the physiochemical characteristics such as zeta potential, average particle size diameter or percentage encapsulation efficiency and percentage loading efficiency of Diclofenac sodium. Chitosan nanoparticles had shown an excellent capacity for the association of Diclofenac sodium. The average particle size range was found between 600-1000 nm with zeta potential of 20mV. The percentage entrapment efficiency, percentage loading efficiency was found to be 92.33±3.05% and 64.90±2.29% respectively and the drug release was found to be 80.6±3.50% due to its physical entrapment. Previous studies on Chitosan nanoparticles have reported encapsulation of several compounds, their *in vitro* release profiles and *in vivo* applications. In this study, we have encapsulated Diclofenac sodium with Chitosan polymer which has not been formulated in a drug delivery system for intra-articular administration.

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