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Chiotsan Nanoparticles Loaded with Thiocolchicoside

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ABSTRACT

The oral administration of thiocolchicoside as skeletal muscle relaxant remains a less effective management of rheumatism and arthritis owing to poor oral bioavailability. Hence the present study was aimed to develop and evaluate nanoparticles containing thiocolchicoside as potential oral drug delivery system. The thiocolchicoside loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). The nanoparticles were characterized by Scanning Electron Microscopy (SEM), Zeta potential analyser, Fourier Transform Infra-red (FTIR) Spectroscopy and X- ray diffraction (XRD). All the prepared formulations resulted in nano range size particles (200 - 700 nm). The entrapment efficiency were found to be 74%- 81% respectively. The thiocolchicoside was found to be dispersed in the nanoparticles in microcrystalline polymorphic form. The invitro release profile of thiocolchicoside from the nanoparticles showed a sustained release of the drug over a period of 5 hrs.

Keyword: Thiocolchicoside , Chitosan, Nanoparticles, Ionic gelation method

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INTRODUCTION

Chitosan (CS) is a naturally occurring nontoxic, biocompatible, biodegradable, cationic polysaccharide. This hydrophilic polymer can easily cross-link with counter poly anions like TPP to control the release of drugs CS is a mucoadhesive polymer with permeation enhancing properties which facilitate opening of the epithelial tight junction[1-2]. Moreover Chitosan nanoparticles can be easily prepared under mild conditions, besides can be incorporated with low molecular weight drugs. This characteristic is extremely beneficial for drugs, proteins, genes or hydrophobic molecules that are poorly transported across epithelia. Among the various methods developed for preparation of nanoparticles, ionic gelation method is simple to operate and also to optimize the required particle size of the drug that can penetrate the epithelial membrane and hence this method was followed in the study. Moreover, chitosan has been proposed as a material with a good potential for oral drug delivery [3-6].

Thiocolchicoside (TH) is N-[(7S)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide, a sulfur derivative of colchicoside). Thiocolchicoside is an agonist of the GABA receptors in the central nervous system, exhibiting muscle relaxation, analgesic and local anesthetic activities. Therefore, thiocolchicoside is prescribed for the treatment of orthopedic, traumatic and rheumatologic disorders. The oral relative bioavailability of thiocolchicoside compared with intramuscular administration, is approximately 25%, mainly due to hepatic first pass-effect, with a high intra- and inter-subject variability [7-9].

MATERIALS AND METHODS

Materials

Thiocolchicoside was obtained as a gift sample from Emcure Pharmaceuticals (Pune, India). Chitosan (degree of deacetylation of 93%;) was purchased from Research Lab. (Mumbai, India). Sodium tripolyphosphate (TPP) was purchased from S.D. Fine Chemicals Ltd (Mumbai, India). All other reagents and solvents used were of analytical grade.

Methods

Chitosan nanoparticles were prepared according to the ionic gelation of Chitosan with Pentasodium tripolyphosphate (TPP) anions. Plain chitosan were used for formulating nanoparticles. Four samples were prepared:

Chitosan was dissolved in 3 % v/v acetic acid aqueous solution at various concentrations 0.5%w/v**(F1)**, 1.0 w/v**(F2),** 1.5%w/v**(F3),** 2.0 w/v**(F4)** . Add 20% NaOH to adjust pH 4.7-4.8. Aqueous solution of TPP 3.3ml with 0.5%w/v concentration was added through a syringe needle into 10ml chitosan solution under stirring at room temperature. The phenomena observed was in aqueous solution. Nanoparticles were formed spontaneously upon incorporation of 3.3ml TPP solution into 10ml chitosan solution. The above nano solutions **F1, F2, F3 and F4.** were adjusted to pH 4.5-4.8. The above solutions were freeze dried by using a laboratory freeze dryer(Christ alpha 1-2 D plus) [10-11].

CHARACTERIZATION OF CS NANOPARTICLES

1) % Yield of Chitosan Nanoparticles

The percentage yields of chitosan nanoparticles were calculated from the weight of dried nanoparticles recovered (W_1) and sum of initial dry weight of starting material (W_2) as:

$$\textbf{Percentage Yield} = \frac{W_1}{W_2} \times 100$$

W_1= weight of dried nanoparticles recovered
W_2= sum of initial dry weight of starting material

2) Entrapment efficiency (EE) of nanoparticles

The nanoparticles suspension (10ml/10ml) were centrifuged at 15000 rpm for 30 minutes. The supernatant solution was separated. 1ml of supernatant was distributed in 10ml distilled water and the absorbance was measured using UV spectrophotometer at 258 nm using water as blank. The amount of drug unentrapped in the supernatant was calculated. The amount of drug entrapped was determined by subtracting amount of free unentrapped drug from total amount of thiocolchicoside taken [12].

The drug entrapment efficiency (EE) of nanoparticles were calculated as follows:

$$\textbf{Entrapment Efficiency (\%)} = \frac{\text{Total amount of drug} - \text{free unentrapped drug}}{\text{Total amount of drug}} \text{ X } 100$$

All measurements were performed in triplicate.

3) Content Uniformity

Content Uniformity was determined by dissolving nanoparticles in phosphate buffers solution (PBS) at pH 7.5 for 5 hrs and UV absorbance was recorded to estimate the % drug content.

4) Surface Morphology

The scanning electron microscope (SEM) is a type of electron microscope that gives images of the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms

that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity. A wide range of magnifications is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 500,000 times, about 250 times the magnification limit of the best light microscopes. The surface topography of the nanoparticles was examined using a scanning electron microscope (Jeol, JSM-6360, Japan, and 15 KV). Samples were coated with platinum film under vacuum using a sputter coater (SPI SputterTM Coating Unit, SPI Supplies, Division of Structure Probe, Inc., PA, USA) and then investigated at 200-1000 nm.

5) Particle size analysis

The particle size distribution of chitosan bound drug nanoparticles was determined by using dynamic light scattering (DLS) using Malvern instrument (mastersizer2000).Dynamic light scattering was equipped with a 35 mw He-Ne laser beam with wavelength of 523nm. All DLS measurements were performed at 25^0C and refractive index and viscosity of water were taken as 1.590 and 0.8904 cp respectively which were used for calculating effective diameter from autocorrelation. Nanoparticles size measurements were carried out by using water as medium.

6) Fourier transform-infrared spectroscopy (FT-IR)

FT-IR spectra were obtained using a Shimadzu spectrometer, model Spectrum 8400S. In order to collect the spectra, a small amount of freeze-dried nanoparticles was mixed with KBr (1% w/w nanoparticles) and compressed to form pellets. The IR spectra of these pellets, in absorbance mode, were obtained in the spectral region of 450–4000 cm^{-1} using a resolution of 4 cm^{-1} and 64 co-added scans.

7) In-vitro Drug release studies in pH 7.5 PBS

7.1) Method I for Capsule:

In-vitro Drug Release studies of thiocolchicoside capsule were carried out by using USP XXIII Dissolution Test Apparatus 1(Basket method) at 37± 0.5^0C and 100 rpm speed. The capsule was placed in the baskets and then submerged into 900 ml dissolution flask containing 500 ml of PBS pH 7.5 for 1 hr. Aliquots of 2.5 ml were withdrawn and replaced with the same volume of fresh solution at each different time intervals. Aliquots withdrawn were filtered through Whatman filter paper number 42. The amount of drug released was analyzed by UV-visible Spectrophotometric assay method. The release studies were conducted in triplicate and mean values of cumulative % drug release were plotted versus time.

7.2) Method II for Nanoparticles

Nanoparticle samples (5 mL), were enclosed in dialysis bags (cellulose membrane, mw cut-off 12400, Sigma), and were incubated in 200 ml release medium at 37 ^{0}C under mild agitation in a water bath. The release medium was phosphate buffer having pH 7.5 in the case of chitosan nanoparticles. At predetermined time intervals, 2.5ml samples were withdrawn from the incubation medium and analyzed for drug release (thiocolchicoside) by UV spectrometry.

8***) X-Ray Diffraction Study***

X-Ray Diffraction was used to study physical form of drug dispersion with chitosan matrix of nanoparticles. X-Ray Diffraction Study was performed in Brucker D 8 Advanced X-ray diffractometer using Cu K 2α ray with a voltage of 40 kV and current of 25mA. Samples were scanned for 2θ from 10 to 60^0. Diffraction pattern for nanoparticles was obtained**.**

9) Stability Studies

To assess the drug and formulation stability, stability studies were done according to ICH guidelines. Optimized formulation sealed in aluminum packaging coated inside with polyethylene, and various replicates were kept in the humidity chamber maintained at 40°C and 75% RH for 45 days. At the end of studies, samples were analyzed for the physical appearance, drug content and drug release studies.

RESULT AND DISCUSSION

1)FT-IR Spectroscopy

For chitosan nanoaprticles, the peak of amide I(-C=O-NH_2) bending shifted from 1635cm^{-1}, and new peaks appeared around 1160cm^{-1} (P–O), implying the complex formation via electrostatic interaction between phosphoric groups

and ammonium ions. In comparison with the FT-IR spectrum of chitosan, drug bound chitosan nanoparticles, showed shift in carbonyl group which got buried in big envelop of 1536 cm^{-1}. The peak at 3267 cm^{-1} become broader indicating hydrogen bonding between thiocolchicoside and chiotsan.

2)Particle Size analysis:-

The particle size and polydispersity (size distribution) optimized batch of chitosan nanoparticles measurement were performed by using Zetasizer (Malvern Instruments, UK) by dynamic light scattering technique. It showed drug loaded nanoparticles were found to be 200-700 nm. The characteristics of the chitosan/TPP particles prepared with different concentrations of chitosan or TPP were studied. The results indicated that the particle size increased with increasing the concentration of either chitosan or TPP.

It is known that under acidic conditions, there is electrostatic repulsion between chitosan molecules due to the protonated amino groups of chitosan; meanwhile, there also exist inter-chain hydrogen bonding interactions between chitosan molecules. Below a certain concentration of LMW chitosan (2.0 mg/mL), the intermolecular hydrogen bonding attraction and the intermolecular electrostatic repulsion are in equilibrium. Therefore, in this concentration range, as chitosan concentration increases, chitosan molecules approach each other with a limit, leading to a limited increase in intermolecular cross-linking, thus larger but still nanoscale particles are formed. Above this concentration, microparticles are easily formed probably due to the stronger hydrogen bonding interactions leading to plenty of chitosan molecules involved in the cross-linking of a single particle. The formation of micro-particles usually leads to a flocculent precipitate as the electrostatic repulsion between particles are not sufficient to maintain the stability of these large particles. The increase in NPs size in the presence of 1 mg/mL thiocolchicoside feeding solution is not clear, but our hypothesis is that enhancing the thiocolchicoside amount and consequent adsorption, a conformational reorganization of the polymer occurs leading to NP swelling.

Table No 1: Percent yield, entrapment efficiency, Particles Size of chitosan nanoparticles

Sr. No.	Batch Code	Chitosan:TPP	Percentage yield	%Drug content (w/w)	EE* (%w/w)	Particle Size (nm)
1.	F1	1:1	83.03	67.05±23	74.04	195
2.	F2	2:1	82.05	78.4.21±56	81.07	285
3.	F3	3:1	77.90	77.10±0.38	78.23	435
4.	F4	4:1	76.90	75.25±0.10	79.23	697

3)Percent yield, entrapment efficiency and drug content.

Results of preliminary investigations on the experimental conditions for the formation of chitosan nanoparticles showed that nanoparticles could be obtained by varying the concentrations of chitosan with concentration of TPP respectively. As concluded in Table No.1 % yield, entrapment efficiency of the nanoparticles was affected by the varying chitosan concentration. This might be due to the concentration of TPP taken as constant to achieve the chitosan: TPP nanoaprticles for effective drug entrapment. As the polymer concentration increases the entrapment efficiency of dug also increases. Thus batch F2 having chitosan: TPP (2:1) ratio was selected for further studies as it showed highest % yield, entrapped efficiency and % drug content.

4) Scanning electron microscopy (SEM)

The morphological characters of thiocolchicoside loaded chitosan nanoparticles (F2) are shown in Figure **1.** Thiocolchicoside loaded chitosan nanoparticles were irregular in shape.

The thiocolchicoside loaded chitosan nanoparticles showed increases in particle size after freeze drying which is found to be common with unmodified chitosan nanoparticles. This has resulted due to aggregation from the strong interaction due to inter- and intra-molecular hydrogen bonding which was not possible to break after sonication.

5)X-ray diffraction studies

From X-ray powder diffraction as shown in Fig. No.2 the internal physical state of thiocolchicoside in solid nanoparticle was further verified. The thiocolchicoside show intense peak at $2\theta = 11.8^0$, 16.9^0, 19.1^0, 22.5^0, 25.1^0, 26.9^0, 29.7^0, 32.6^0, 41^0, 43.9^0, 44.6^0. Indicating crystalline structure of drug as shown in following Fig. No. 2 . The diffraction peaks of thiocochicoside on its nanoparticles with chitosan were not found at same possition as shown in following Fig. No. 3. Instead, new peaks appeared at 2 θ = showed at 12.6^0, 13^0, 17.3^0, 17.8, 20^0, 22.9^0 , 25.5 with low intensity. The new peaks show microcystalline polymorphic modification of Thiocolchicoside**.**

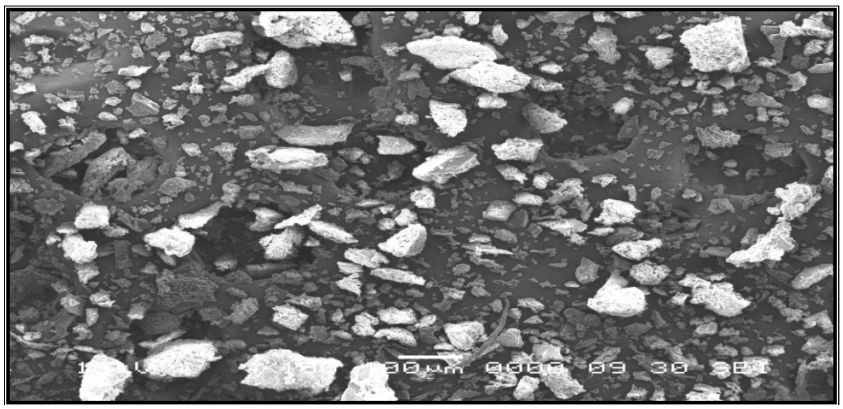

Figure no.1: SEM images of chitosan nanoparticles

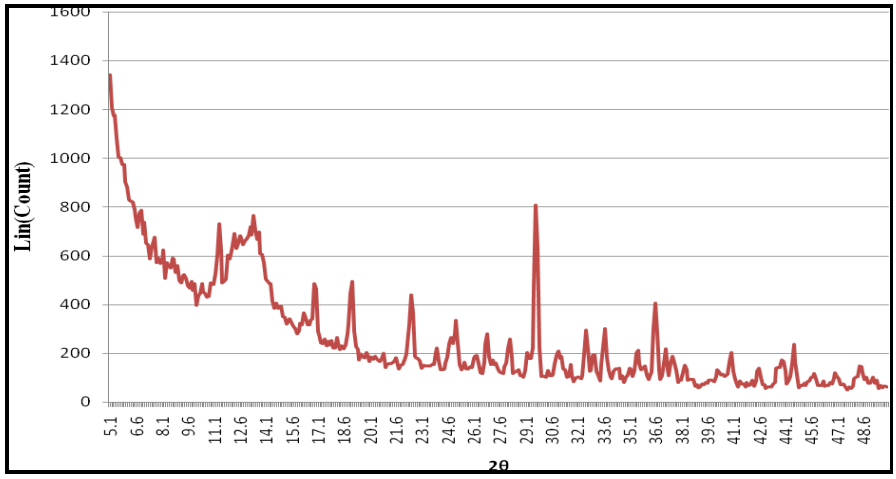

Figure no. 2: XRD patterns of Thiocolchicoside

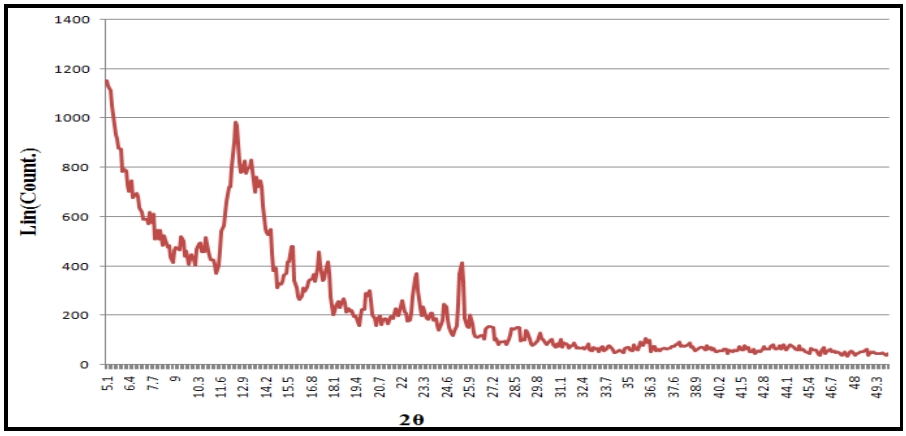

Figure no. 3: XRD patterns of nanoparticles formulation of Thiocolchicoside.

6)In-vitro Drug release studies

6.1) % release of Thiocolchicoside from Marketed Formulation :-

The release profiles in PBS pH 7.5 thiocolchicoside capsule is depicted in Fig.No.4. Release of thiocolchicoside was found to be about 92.45% within 40 minutes. There is immediate release of drug from marketed formulation of thiocolchicoside capsule (Fig.No.4).

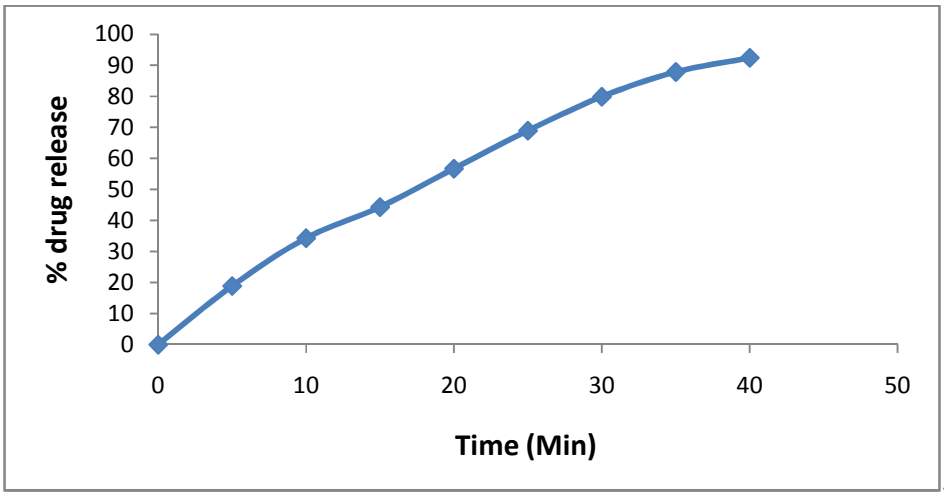

Figure No. 4: In vitro % release of Thiocolchicoside release in pH 7.5 from marketed formulation

6.2) % release of Thiocolchicoside from loaded chitosan nanoparticles :-

The release of thiocolchicoside from loaded chitosan nanoparticles in PBS pH 7.5 is as depicted in Fig. No.5. Thiocolchicoside release of about 84.27%, was observed within 270 minutes. As the concentration of polymer increases the sustained release time for Thiocolchicoside increases.

Thiocolchicoside release from nanoparticles takes place by several mechanisms including surface erosion, disintegration, and diffusion and desorption mechanisms. The release profiles of thiocolchicoside from thiocolchicoside loaded chitosan nanoparticles were investigated in vitro at ambient condition for 4-5 hrs. Thiocolchicoside from the nanoparticles follows a biphasic pattern, characterized by an initial rapid release period (burst release) followed by a period of slower release. The burst release lasted 45 minutes, and during this period approximately 35-45% was released and this was followed by a period of much slower release for the rest amount of drug going up to 90% in 4.5 hrs. For all the nanoparticle batches.

The marketed formulation of thiocolchicoside capsule showed quick release as compared to nanoparticles. Nanoparticles showed sustained drug release effect in-vitro, where as marketed formulations of thiocolchicoside showed immediate drug released effect.

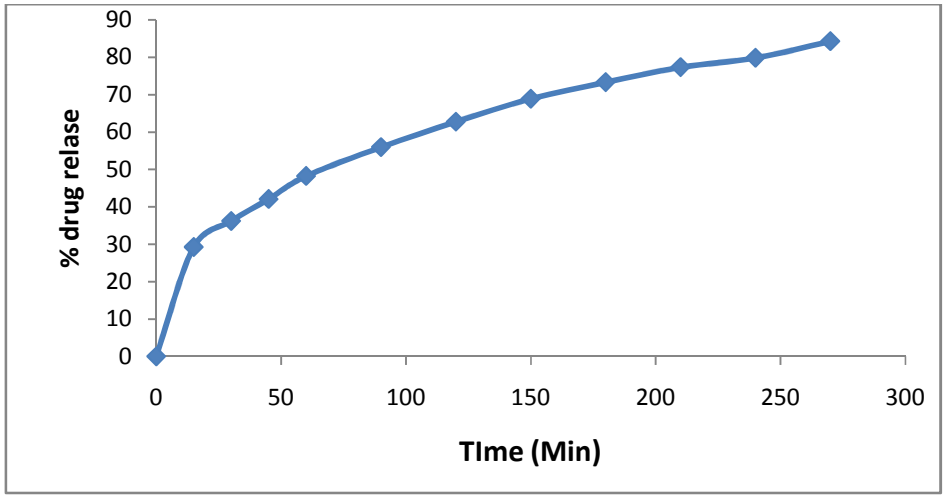

Figure No. 5: In vitro % release of Thiocolchicoside loaded chiotsan nanoaprticles in pH 7.5 from F 2

7)Stability Studies

The stability studies were carried out on optimized dried nanoparticles. The formulations were stored at $40\pm2^0/75\pm5\%$ RH (climatic zone IV condition for accelerated stability testing as per ICH guidelines) for one month in hard gelatin shell to assess their stability. After 15 and 30 days samples were withdrawn and retested for physical appearance, % drug content and % drug release as shown in Table No 2.The result indicated that formulations were able to retain their stability for 30 days without significant degradation.

Table No 2: Stability studies data of Batch F2

Day	Solid nanoparticles		
	Appearance	% content	% drug release
0	Slight yellowish	100±0.32	80.91±23
15	Slight yellowish	98.8±0.23	79.87±78
30	Slight yellowish	97.90±0.90	81.13±18

CONCLUSION

The thiocolchicoside loaded nanoparticles were successfully prepared by cross linking with TPP. Drug: polymer ratio had an impact on entrapment efficiency and % drug release from nanoparticles up to certain level. Further increase in polymer concentration had significant effect. Increase in stirring speed help to some extent to decrease the particle size, entrapment efficiency and sphericity. Out of above batches, F2 was selected for further studies. Nanoparticles were evaluated for % content uniformity and entrapment efficiency. IR spectral analysis was also studied to confirm the entrapment of thiocolchicoside in the nanoparticles. Based upon XRD data, thiocolchicoside was found to be dispersed in the nanoparticles in microcrystalline polymorphic form. This was probably due to potent interaction developed between thiocolchicoside and CS/TPP matrix as revealed in FT-IR data. The release profile of thiocolchicoside from nanoparticles has an initial burst effect followed by slow continuous release as compared to marketed formulation. Stability study of the optimized batches showed that the nanoparticles were stable for 30 days.

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