Betamethasone and Ketoprofen Nanoparticles: Optimization, Evaluation and Anti-inflammatory Activities for Osteoarthritis

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Abstract

The nanoparticle contains Ketoprofen and Betamethasone used for decreasing inflammation and simultaneously inhibits the transcription of Interleukin-12b (Il12b) in microphages. The purpose of this research is to develop mucoadhesive formulation of chitosan and sodium alginate nanoparticle of betamethasone and ketoprofen using the ionotropic gelation process. Prepare nanoparticles were proceeding for the physicochemical characterization like zeta potential, Particle size, morphology, entrapment efficiency, in-vitro release, FTIR and stability. Further, the anti-inflammation activity was confirmed by In-vitro anti-inflammatory macrophages test. Depending on the formulation circumstances, the mean particle size of selected batch (F6) was 145.7±7.6 nm and 79.8 ± 6.1% encapsulation efficiency was recorded. The particle displayed smooth surface and no interaction between the excipients and drug. The in vitro release experiments demonstrated a quick burst release of the medication initially followed by a slow sustained release at different time interval of 24, 48, or 72 hours and proved good stability at both 25°C and 40°C. Also the nanoparticles inhibited hemolysis to a degree ranging from 3.8% to 11.1%. According to the study's findings, betamethasone and ketoprofen may be delivered as a sustained release manner using chitosan alginate nanoparticles.

Keywords: Betamethasone, Ketoprofen, nanoparticles, chitosan, inflammation

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Introduction

Osteoarthritis (OA) is a prevalent chronic joint disease marked by stiffness and discomfort in the joints, as well as a restriction in range of motion. It is the leading cause of adult disability. Age, gender, obesity, joint damage, and other factors are associated with the incidence of OA. Due to its great incidence, OA places a tremendous financial burden on both society and individuals. The pathogenesis of OA is characterized by sclerosis, osteophyte production, synovial inflammation, subchondral bone remodelling, and gradual and damage of articular cartilage gradually. This condition is thought to be a complicated disease involving numerous tissue and process. The root causes are still not completely known [3]. As a result, it has proven challenging to pinpoint precise therapeutic targets. Instead of focusing on the mentioned causes of OA at molecular level, recent practice is clinically largely restricted to symptomatic relief aimed at pain management, improvement in function, and sometimes joint replacement.

The elastic connective tissue called articular cartilage, which covers the ends of the bones, aids in the smooth motion of joints. The articular cartilage and synovial membrane contains synovial fluid, fills the joint cavity. Two crucial chemicals, hyaluronan and lubricin, are secreted by the fibroblasts and macrophages that make up the synovial membrane. They help lubricate the articular cartilage's border and increase the viscosity of synovial fluid. Proteolytic enzymes, pro-inflammatory cytokines, and reactive oxygen species (ROS) are the three main biological mechanisms that contribute to and aggravate cartilage breakdown in OA. Moreover, OA is associated to altering degrees of inflammation in synovial that accelerate by cartilage degradation and impairment. Cytokines, protease, chemokines and reactive oxygen species release by the chondrocytes, macrophages and synovial fibroblast promote the OA worsens. Synovitis, chondrocyte viability is increased by interleukin-1 (IL-1) and tumor necrosis factor (TNF) and provoked the synthesis of many proteases.

An interdisciplinary area of science for research and education is nanotechnology. Due to their scale structure, nanoparticles (NPs) frequently exhibit singular characteristics, such as size

effects, interfacial phenomena, and quantum effects, giving them a variety of benefits and innovative functionalities. Osteoarthritis therapy by intravenous and intra-articular injection, extensive range of drug delivery system of nanoparticle, micelles, liposomes, polymeric nanoparticles, inorganic NPs, dendimers including polymer have been studied. These nanotherapeutic approaches enhance drug targeting, effective drug delivery, drug solubility and drug stability. Also, they lengthen medication circulation and retention time, hold up the dispersion of drug and inclination in body fluids, and improve treatment efficacy by minimizing negative drug reactions. Our study's goal was to create flexible nanoparticles that were simultaneously loaded with Betamethasone and Ketoprofen for the treatment of Osteoarthritis. The nanoparticles physicochemical characteristics, including average particle size, size distribution, morphological observation, in vitro drug release, FTIR, stability and in-vitro anti inflammation were comprehensively described.

Materials and methods

Betamethasone (betamethasone sodium phosphate) and Ketoprofen were obtained from the HiMedia Laboratories. Chitosan and tween 80 were obtained from the Sigma Aldrich. The sodium alginate was purchased from IndiaMART. Lactic acid and calcium chloride were procured from SD fine chemical, India PVT.LTD.

Methods

Identification of Betamethasone and Ketoprofen

The both drug Betamethasone (BSP) and Ketoprofen were analyzed by UV spectrophotometer applying simultaneous estimation method. BSP was dissolved in distilled water to create a BSP solution and prepared a different dilution. When the solution's UV absorbance was scanned, the absorbance peaked at 246 nm. By measuring the absorbance of various BSP solution concentrations, a calibration curve was produced (11).

Similarly, 10 mg ketoprofen was accurately weighed and dissolved in distilled water. The solution was placed into a 100ml volumetric flask after shaking vigorously for 10 minutes. The volume was finally finished, marked, and filtered. The resulting solution was proceeded for further dilution. At 260 nm, the absorbance values were calculated. Direct sample/standard comparison or a calibration curve were both used to calculate the content for each drug (12).

Formulation of Betamethasone and Ketoprofen nanoparticles

The solutions of chitosan and sodium alginate were made by combining the polymers with distilled water. The desired concentrations of chitosan were dissolved the required quantity of chitosan in solution of 1% lactic acid. In order to achieve the requisite concentrations, solution of calcium chloride were made by using the necessary quantity of calcium chloride in distilled water. The preparation process for the nanoparticles is called ionotropic-gelation. The process for making alginate-poly-Llysine nanoparticles involves two steps:

In first step involved creation of calcium alginate pre-gel is the initial step in the nanoparticles preparation process, in which, calcium chloride solution was added with stirring in 10 ml solution of sodium alginate at a 400 rpm speed (1, 13). In second step, added the 6 ml solution of of chitosan in continuously stirred calcium alginate pre-gel. To enable nanoparticles to form uniform particles, the resulting opalescent suspension was allowed to equilibrate at room temperature for an entire night. These circumstances were employed while loading the nanoparticles with the drug. The different ratio (1:1, 1:2 and 2:1) of Betamethasone and Ketoprofen were dissolved in the sodium alginate solutions.

Adding 9 ml solution of calcium chloride to 15 ml solution of sodium alginate including the different ratio of drug with stirring at 400 rpm for 1.5 hours. The chitosan solution was then added in 6 ml throughout the course of the following 1.5 hours with constant stirring. Overnight equilibration was performed on the resulting opalescent dispersion to promote homogenous nanoparticle formation.

The impact of several experimental variables like % calcium chloride and % sodium alginate concentrations, % chitosan were studied in formulation parameters like particle size and entrapment efficiency. To prevent nanoparticle aggregation a non ionic surfactant solubilizing agent, tween 80, was added. Tween 80 was studied at three different concentrations (0.5, 1 and 1.5%) to see how it affected the physical and chemical characteristics as well as the in vitro release. The made-up formulations' composition is displayed in Table 1 below.

Table 1: The formulation development of chitosan nanoparticles with evaluation parameter particle size and entrapment efficiency

Formulation code	**% Sodium**	**% Calcium**	**Tween 80**	**% chitosa**	**Ratio of Betamet**	**Particle size**	**Entrapment efficiency**

	alginate	**chloride**		**n**	**hasone and Ketoprofen**		
F1	1.5	1.0	0.5	0.5	2:1	245.5±7.6	64.9 ± 1.2
F2	2.0	1.0	0.5	0.5	1:2	175.7±4.0	54.3 ± 6.7
F3	2.5	2.0	0.5	0.5	1:1	195.7±8.1	60.5 ± 1.2
F4	3.0	2.0	0.5	0.5	2:1	205.5±1.9	65.1 ± 5.2
F5	1.5	2.0	1.0	1.0	1:2	240.7±3.5	71.4 ± 3.5
F6	2.0	2.0	1.0	1.0	1:1	145.7±7.6	79.8 ± 6.1
F7	2.5	1.0	1.0	1.0	2:1	135.1±4.9	57.9 ± 9.3
F8	3.0	1.0	1.0	1.0	1:2	215.3±8.2	64.5 ± 0.8
F9	1.5	1.0	1.5	1.5	1:1	302.4±6.1	54.3 ± 6.4
F10	2.0	1.0	1.5	1.5	2:1	125.6±5.2	61.6 ± 8.2
F11	2.5	2.0	1.5	1.5	1:2	165.7±8.4	56.4 ± 5.1
F12	3.0	2.0	1.5	1.5	1:1	112.8±3.9	70.8 ± 3.5

Characterization of nanoparticles

Average particle size and size distribution

Dynamic light scattering method (Zeta potential/particle sizer NIOCOMP 380 ZLS, USA) at 25°C, at detection angle of 90°, was used for determining the average particle size and size distribution. Nanosuspension of 1 ml was added in distilled water to make 10 ml before analysis. Same apparatus was used for the zeta potential estimation. Taken the sample of 10 ml and centrifuged, removed the supernatant, in order to determine the zeta potential of the particles (14,15).

Morphology of chitosan-alginate nanoparticles

On a copper grill, chitosan-alginate nanosuspension containing drug solution of 1 ml was applied, and it was allowed to air dry. Transmission electron microscopy was used to examine the nanoparticles' morphology (Joel 100CX, Japan).

Determination of the encapsulating efficiency

The determination of encapsulating efficiency of nanoparticles, firstly taken the sample of 10 ml was centrifuged at 8000 rpm for 30 min. After that collected the supernatant and take step for the UV spectrophotometry analysis at 246 nm and 260 nm using simultaneous estimation method (16). The absorbance was calculated after diluting one millilitre of the supernatant to 100 millilitres. The given equation was used to determine the encapsulating efficiency:

Encapsulating Efficiency = [(Total amount of drug- free drug)/ Total amount of drug] X 100

In vitro release

Cellophane membrane was used for in vitro release study of the selected formulations. The 1ml developed formulation were filled in presoaked cellophane membrane and properly tight with both the end. The formulation filled cellophane membrane was kept in beaker which contain 100ml of PBS that had been preheated to 37°C. Receptor compartment was constantly agitated with magnetic stirrer at 200 rpm. Sample solution of 1 ml was taken and refilled with 1 ml of fresh solution, PBS at specified intervals of time (0.25, 0.5, 1, 2, 3, 4, 5, 6, 24, 48, and 72 hours). Using a UV spectrophotometer at 246 nm and 260nm, the drug content was tested by withdrawn sample, placebo chitosan-alginate nanoparticles used as a base of correction (17,18). The outcomes of an in vitro drug release study were fitted with given kinetic equations, including Higuchi's model (cumulative % drug release vs. square root of time), zero order (cumulative% drug release vs time) and first order (cumulative log % drug remaining vs time).

FT-IR analysis

To determine whether chitosan/alginate, Betamethasone and Ketoprofen interact, FT-IR analysis were done. The Nicolet Avatar 380 spectrometer in the USA was used; FT-IR spectra were captured. Mainly chitosan, sodium alginate, Betamethasone, Ketoprofen and nanoparticles of Betamethasone and Ketoprofen were obtained (19).

Stability Studies

The stability of prepared chitosan-alginate loaded nanoparticles was assessed, after being stored for three months at various temperatures. Betamethasone and Ketoprofen nanoparticles were kept at 25° 2°C or 40° 2°C away from light in polyethylene plastic bottles with droppers. Each

sample had Benzalkonium chloride (0.02%) added as a preservative to stop microbial development while it was being stored. Samples were taken out at different time interval at 1,2 and 3months and analyze for particle size and encapsulating effectiveness (20).

In vitro anti-inflammatory activity

The human whole blood was drawn and collected in centrifuge tubes containing heparin. The blood was spun for 5 min at 3000 rpm before being rinsed three times with saline (0.9% NaCl). For preparation of suspension, isotonic buffer solution (10mM sodium phosphate buffer pH 7.4) of 10%(v/v) was added in whole blood sample and centrifuged (21). The different concentration (25, 50, 75, 100µg/mL) of drug-loaded nanoparticles was prepared with above suspension. The mixture was incubated in water bath that was shaken at 54°C for 20 min. Following incubation, the mixture was centrifuged for 5 min at 3000 rpm and the absorbance of supernatant at 540 nm was assessed by UV/VIS spectrophotometer (Optima, SP-3000, Japan). For the experiment, solution of phosphate buffer served as the control and Diclofenac served as the reference drug (22). The following equation was used to determine the % hemolysis:

% Hemolysis = (Absorbance of test sample/ Absorbance of control) X 100

Result and discussion

The different batches of Betamethasone and Ketoprofen loaded chitosan nanoparticles were successfully prepared. On the basis of encapsulating efficiency, zeta potential and particle size were selected. The concentration of calcium chloride and sodium alginate is increased resulted in larger particles and less effective encapsulation. This can be explained by the fact that calcium chloride and sodium alginate make up the majority of the nanoparticle matrix, leaving less space for the drug encapsulation. The findings are in line with earlier research that demonstrated that as alginate or chitosan concentrations rise, particle size increases and encapsulating efficiency decreases. The concentration of sodium alginate was 1%, results in a negative zeta potential because chitosan amount became too less to interact with all carboxylic group of alginate alginate, This occurred because a large amount of alginate formed the core of the nanoparticles.

Several chitosan concentrations (0.5, 1 and 1.5%) were prepared. Particle size and encapsulating effectiveness were both impacted by the variation in chitosan concentrations. The alginate

became the limiting reactant, when the concentration of chitosan was increased and cause precipitation of unreacted chitosan molecule resulted in formation of clumps (group together). As some molecules of chitosan adhere to the chitosan-alginate nanoparticles surface, the particle size increased unevenly. Despite the fact that the particle size was increased, the encapsulating efficiency somewhat decreased as chitosan concentration increased. This occurs as a result of non-uniform particle size distribution in this instance. However, the impact on encapsulating efficiency was minimal.

Several drug ratios (1:1, 1:2 and 2:1) were generated in order to investigate the impact of drug concentration (Betamethasone and Ketoprofen) on size of particle and encapsulation effectiveness. The particle size and encapsulation effectiveness decreased as drug concentration was raised. Drug potential is increased into the external solution for diffusion, is the reason of reduction in encapsulating efficiency with increasing drug content in sodium alginate and original mixture. In accordance with earlier research, adding more dexamethasone to chitosan tripolyphosphate nanoparticles reduces the loading efficiency because the medication diffuses into the external solution. The particle size reduced as the drug's concentration was raised. In order to encapsulate the drug, more nanoparticles with smaller particle sizes were formed when the drug concentration was higher. When the amount of medication contained within the nanoparticles increased, more chitosan was drawn to the superficies of the alginate nanoparticles due to an increase in the negative charge. This raised the zeta potential and positive charge on the superficies of nanoparticle of chitosan alginate. Dynamic light scattering method was used to determine particle size and varied from 112.8±3.9 to 245.5±7.6 nm depending on the experimental conditions. The selected batch F-6 shown the 145.7±7.6 nm and 79.8 ± 6.1% entrapment efficiency.

Morphology characterization

Transmission electron microscopy (TEM) analysis of the produced nanoparticles revealed that they were solid dense structure, spherical particles and distinct. The nanoparticles, however, had a fluffy look rather than a smooth surface (Figure 1).

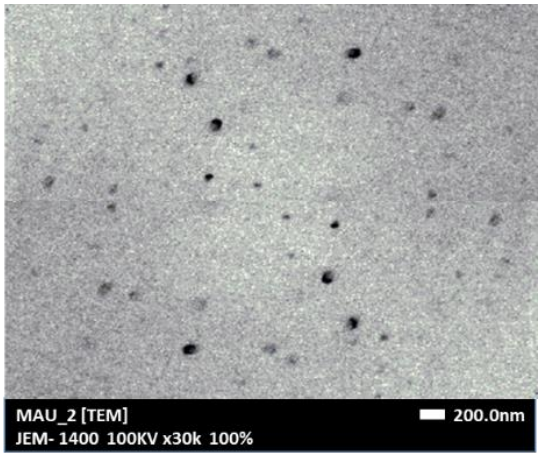

Figure 1: TEM image of chitosan particles

In vitro release

Triplets were used in studies on in vitro drug release. Selected formulation was proceeded and samples were taken at various time points, and total drug release percentage was computed. The data of release study was fitted to Higuchi diffusion model and models with zero order and first order (23). Depending on the formulation parameters, the data of drug release study from chitosan alginate nanoparticles (batch F6) abide by zero order (r^2 = 0.9832), first order (r^2 = 0.9973), or Higuchi diffusion (r^2 = 0.9973).

In some formulations, the medication was released in two stages. The formulations demonstrated a rapid initial release followed by a gradual release. The available drug in the nanosuspension and the nanoparticle surfaces were the sources of the initial burst of released Betamethasone and Ketoprofen. At various times, the formulations released between 90 and 100 percent of the medication.

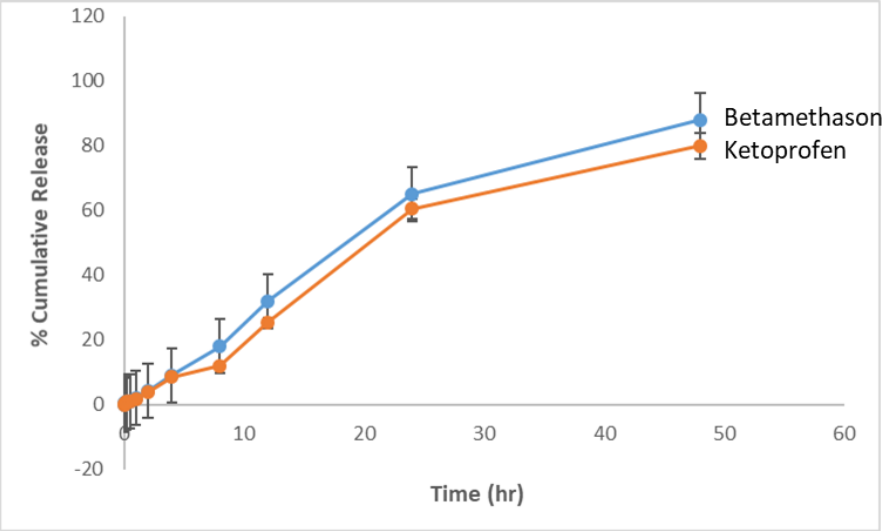

Figure 2: The in vitro release profile of Betamethasone and Ketoprofen chitosan nanopartilces

FT-IR spectroscopy

To describe the probable interactions between nanoparticles, FT-IR was used. Figures 3 depict the FT-IR spectra of betamethasone, ketoprofen, chitosan, alginate and betamethasone ketoprofen loaded chitosan-alginate nanoparticles (figure 3).

The broad band at 3353 cm-1 in the chitosan spectrum was associated with the amine and hydroxyl groups, the peak at 2867 cm-1 was brought on by stretching of the -OH group, the absorption band of carbonyl group (C=O) secondary amide at 1625 cm-1, and the bending vibrations of N-H at 1591 cm-1. N-H stretching of the amide and ether bonds is responsible for the peaks at 1450 and 1382 cm-1, respectively. The secondary hydroxyl group (typical peak of CHOH in cyclic alcohols, C-O stretch) and primary OH were the peaks that were seen at 1060 and 1022, respectively (24).

Alginate's saccharide structure is thought to be responsible for the bands near 1022 cm-1 (C-O-C stretching) in its spectra. The stretching peaks of carboxylate salt groups are allocated to the bands at 1593 and 1402 cm-1 in addition.

The band around 3100 to 3500 cm-1 becomes broad in the IR spectrum of betamethasone sodium phosphate-loaded chitosan alginate nanoparticles, indicating that hydrogen bonding is

increased. The N-H bending vibration of unacetylated 2-aminoglucose primary amines (band at 1591 cm-1) and asymmetric and symmetric -C-O stretching at (1592 and 1402 cm-1) are other indicators of this enhancement (25).

The betamethasone ketoprofen loaded chitosan alginate nanoparticles showed some distinctive betamethasone absorption bands, indicating that betamethasone ketoprofen was filled in the polymeric network. Some bands, however, vanished as a result of the drug and polymer hydrogen interaction. According to these findings, a polyelectrolyte complex is created when ammonium groups of chitosan and carboxylic groups of alginate contact electrostatically.

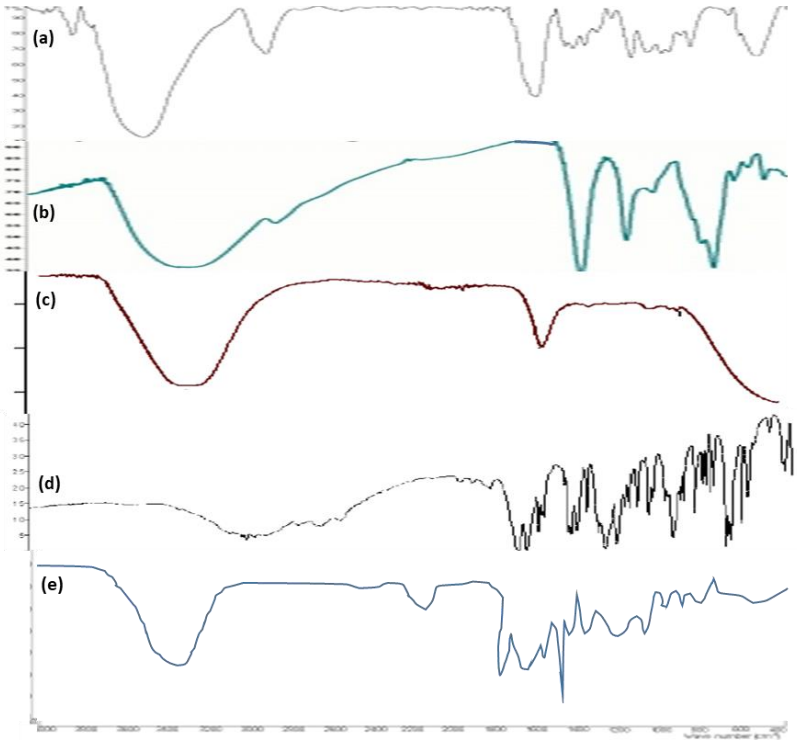

Figure 3: FTIR spectra of (a) chitosan, (b) sodium alginate, (c) Betamethasone, (d) Ketoprofen and (e) nanoparticles of Betamethasone and Ketoprofen

Stability study

The selected formulations F6, underwent stability investigations because they displayed favorable outcomes relating to particle size, encapsulating effectiveness and in vitro drug release. For three months, selected formulation was kept at 25°C and 40°C. After three months'

negligible effects were observed in formulation i. e. slightly increases of particles size (151.6±8.3) and slightly decreases the entrapment efficiency (76.4±3.6).

In vitro anti-inflammatory activity

The percent inhibition of betamethasone ketoprofen loaded chitosan alginate nanoparticles in different concentration (25-100µg/mL) is exhibited in figure 4. Hemolysis was inhibited by betamethasone ketoprofen loaded chitosan alginate nanoparticles in a concentration dependent aspect. At the concentration of 25-100 g/mL, theses chitosan alginate nanoparticles inhibited hemolysis to a degree ranging from 3.8% to 11.1%.

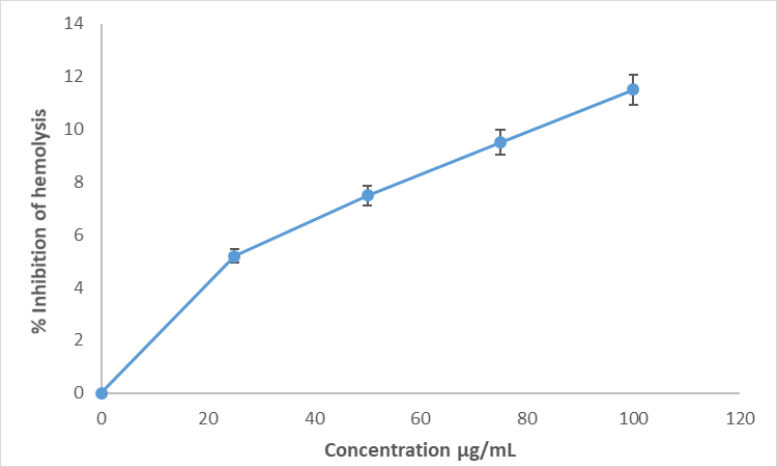

Figure 4: degree of hemolysis at different concentration (25–100 g/mL) of chitosan alginate nanoparticles.

Conclusion:

The outcomes of the study unequivocally demonstrated that nanoparticles of chitosan are a unique drug delivery system with high levels of trapping and sustained drug release over a long duration. The amount of chitosan and presence of sodium alginate had a significant impact on the overall functionality of nanoparticles. Prepared nanoparticle showed desired particles size, entrapment efficiency, and stability. No interaction was observed between the excipients and drug. The hemolysis property of nanoparticles is within the range like to standard drug. Thus, betamethasone ketoprofen loaded chitosan nanoformulation is a potential carrier system and appears to increase the anti-inflammatory efficacy for local effect.

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Conflicts of Interest: The authors declare no conflict of interest.

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