Ketorolac Tromethamine Loaded Chitosan Nanoparticles as a Nanotherapeutic System for Ocular Diseases

Göz Hastalıkları İçin Nanoterapötik Sistem Olarak Ketorolak Trometamin Yüklü Kitosan Nanopartiküller

Research Article

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ABSTRACT

This study mainly focused on the preparation and characterization of Ketorolac tromethamine (ketorolac) loaded chitosan nanoparticles to improve novel drug carrier for the treatment of different ocular diseases such as pseudophakic cystoid macular edema, allergic conjunctivitis and diabetic macular edema. For this purpose; first bare chitosan nanoparticles were prepared ionic co-precipitation technique. Prepared ketorolac loaded chitosan nanoparticles were characterized in three main part of the studies. First morphological evaluations were performed by using Atomic Force Microscope (AFM) and zeta sizer, in second part physicochemical characterization were performed with Fourier Transform Infrared Spectroscopy (FTIR) and finally drug loading-release studies were performed to get different formulations. At the end of the studies, 180-200 nm average size in diameter of the ketorolac loaded chitosan nanoparticles could be produced and proved the ketorolac loading with rather high loading efficiency (around 50 %). Additionally different release profiles could be obtained by using different amount of initial ketorolac concentration during the formulation.

Key Words
Ketorolac Tromethamine, Chitosan Nanoparticles, Drug Delivery, Nanotherapeutic

ÖZET

Bu çalışmanın temel amacı, psedofakik kistoid maküler ödem, allerjik konjuktivit, diabetik maküler ödem gibi çeşitli göz hastalıklarının tedavisi için ketorolak trometamin yüklü kitosan nanopartiküllerin geliştirilmesi ve karakterizasyonunun yapılmasıdır. Bu amaçla, önce iyonik çöktürme yöntemiyle boş kitosan partiküller hazırlanmıştır. Sonrasında ketorolak yüklenmiş kitosan nanopartiküller üç temel kısımda karakterize edilmiştir. İlk kısımda Atomik Kuvvet Mikroskobu (AKM) ve zeta sizer kullanılarak morfolojik değerlendirmeler yapılırken, ikinci kısımda Fourier Transform Infrared Spektroskopi (FTIR) kullanılarak fizikokimyasal incelemeler yapılmış, üçüncü kısımda ise farklı formülasyonlardan elde edilen salım profilleri incelenmiştir. Çalışmaların sonucunda boyutları ortalama 180-200 nm aralığında değişen ketorolak yüklü kitosan nanopartiküller sentezlenmiş ve oldukça yüksek bir oranda ilaç yükleme verimi (% 50 düzeylerinde) elde edilmiştir. Ayrıca, başlangıçtaki ketorolak konsantrasyonuna bağlı olarak farklı salım profilleri elde edilmiştir.

Anahtar Kelimeler
Ketorolak Trometamin, Kitosan Nanopartikül, İlaç Taşınımı, Nanoterapötik

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INTRODUCTION

Various inflammatory ocular conditions have been treated with corticosteroids for more than fifty years [1]. Triamcinolone has been injected directly into the vitreous with the hope of increasing local concentration and duration of therapeutic activity in recent years. Reported side effects of intravitreal steroids include cataract development, increased intraocular pressure, endophthalmitis, retinal detachment, vitreous hemorrhage, and pseudohypopyon [2-5]. One of the alternatives that has been proposed is intraocular nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs inhibit cyclooxygenase enzymes and, thereby, the synthesis of endogenous proinflammatory prostaglandins. They have been used effectively for treating postoperative inflammation and cystoid macular edema and for inhibiting angiogenesis [6,7]. Since topical administration of NSAIDs does not deliver appreciable drug quantities to the posterior segment [8], intravitreal administration of NSAIDs remains a viable option for treating posterior segment disease. However, repeated intravitreal injections entail a risk of retinal detachment and endophthalmitis. A possible alternative to frequent injections of drug solutions is the development of sustained release systems or molecules with prolonged half-lives to reduce injection-related complications, provide enhanced duration of drug effects, and reduce any high-peak-concentration–related side effects encountered with the solution form of the drug [9].

Ketorolac tromethamine (ketorolac) which is commercially available in a preservative-free formulation, and that has been commonly used topically to treat ocular diseases such as pseudophakic cystoid macular edema for more than 10 years [10] is one of these alternatives. It is a nonsteroidal antiinflammatory whose mechanism of action is thought to be principally inhibition of cyclooxygenase [9]. It has been used as a 0.5% topical ophthalmic preparation for several ocular inflammatory conditions such as chronic aphakic and pseudophakic cystoid macular edema [11], allergic conjunctivitis [12] and post-cataract surgery inflammation [13]. Intraocular ketorolac has been shown to significantly reduce inflammation and prostaglandin production in an animal model of uveitis and to have good penetration into the retina but short half-life [14]. Drug delivery systems for providing safer application and longer duration of action for ketorolac may be useful in many ocular conditions where steroid use is precluded due to severe side effects.

This study was aimed to prepare and characterize chitosan nanoparticles loaded with commercially available ketorolac, a NSAIDs whose mechanism of action is thought to be principally inhibition of cyclooxygenase for ocular use.

EXPERIMENTAL

Materials

Chitosan with low molecular weight (i.e., 150 kDa) was obtained from Fluka (Switzerland). Aqueous acetic acid (Glacial/27225/USA) solutions were used as solvent for the chitosan. Sodium tripolyphosphate (Alfa Aesar/013440/USA) was used as cross-linker. Phosphate Buffer Saline, MES buffer [2-(N-morpholino) ethane sulfonic acid and $C_6H_{13}NO_4S$] and glycine (pH 8) were purchased from Fluka (Switzerland). All other chemicals were reagent grade and used without further purification.

Preparation and Characterization of Chitosan Nanoparticles

Chitosan nanoparticles were prepared by using co-precipitation technique as reported elsewhere [15]. In a typical procedure; chitosan nanoparticles were prepared as follows; first, 0.5 g chitosan was dissolved in 0.1% acetic acid. The pH of the solution was adjusted to 4.6 - 4.8 by using 1 M NaOH solution. Then 2 ml of tripolyphosphate (TPP) solution was added to 6 ml pH=4,5 chitosan drop by drop on a magnetic stirrer (1100 rpm). Nanoparticle formation waited for 1 hour, then nanoparticles centrifuged for 15 minutes min at a speed of 9000 rpm and washed by removing supernatant and adding distilled water.

Morphological evaluations of chitosan nanoparticles were realized with an Atomic Force Microscope (AFM, Nano Magnetics Instruments, Turkey). The size of the chitosan nanoparticles were measured by using a zeta-sizer (Nano-ZS Malvern, USA).

Physicochemical characteristics of free Ketorolac, chitosan nanoparticles and Ketorolac loaded chitosan nanoparticles analyzed with FTIR.

Ketorolac Loading and In Vitro Release Studies

Chitosan nanoparticles were formed and dispersed in 1 ml of 4 mg/ml ketorolac tromethamine (Acular Ls, Allergan, USA), then centrifuged and washed for 3 times. Supernatants were analyzed with nanodrop UV visible spectophotometer (Thermo Scientific, USA) and free ketorolac amounts were measured. Loading efficiency was calculated as it is shown in Equation 1.

$$\% \text{ Loading Efficiency} = \frac{\text{Total Ketorolac - Free Ketorolac}}{\text{Total Ketorolac}} \times 100$$

In in vitro release studies Ketorolac loaded nanoparticles were dispersed in 2 ml of Phosphate Buffered Saline (PBS) and placed into the experimental set up. This set up was bult up with 2 ml each of two tubes and the tubes were separated with a dialysis membrane having smaller in pore size as the cut off value (i.e., 12-14 kDa) than nanoparticle average size to keep the drug loaded nanoparticles in the particle side only. This pore size of the membrane permits only the penetration of the drug molecules throughly the membrane pores towards to the other side of the membrane. Released drug was measured with a Nanodrop device (Nanodrop 1000, Thermo Sci., USA) at 324 nm with the sample getting from the nanoparticle free part of the release tubes. Drug release data were obtained in a particular time intervals (i.e., 1, 3, 5, 20, 24, 48 h etc.) by measuring the drug content of the pipetted solution. The pipetted out of the solution was discarded and the same amount of fresh PBS solution was added into the tubes as refreshing the medium to get the sink conditions for drug release.

During the drug loading and release studies, initial amount of drug was changed (i.e., 0.8, 1.6 and 3.2 mg) to get different release profiles and all the release studies were repeated.

RESULTS AND DISCUSSION

Morphological and Physicochemical Evaluations

In this study, chitosan nanoparticles were synthesized with co-precipitation technique and ketorolac was loaded successfully to these nanoparticles. Morphological evaluation of the obtained nanoparticles have shown that sphere like in shape nanoparticles could be prepared and the average size of the nanoparticles was in the range of 180-200 nm with a broad size distribution as shown in zeta sizer output (Figure 1). However, AFM results were shown that a small portion of the nanoparticles are smaller than 100 nm in size and aggregation the nanoparticles due to the sampling procedure (Figure 2). Also,

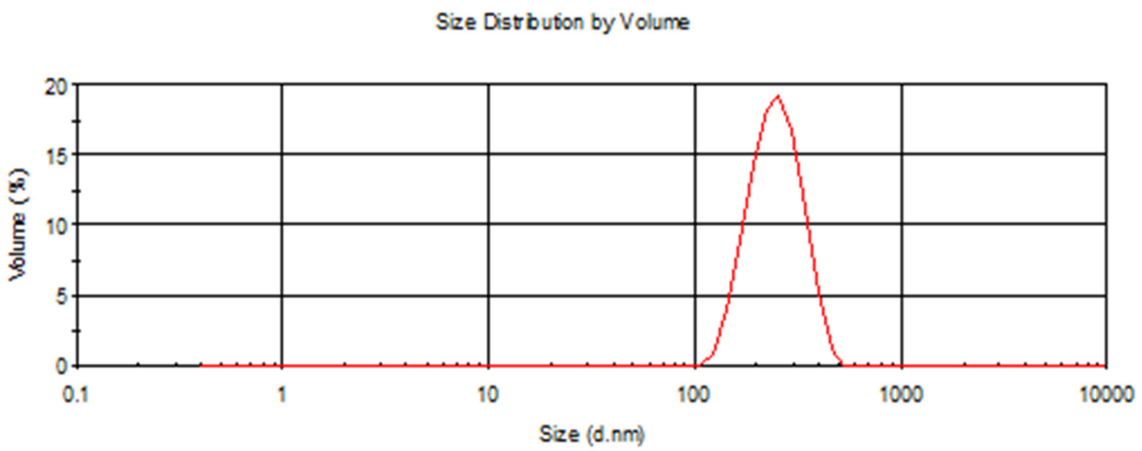

Figure 1. Zeta sizer diagram for chitosan nanoparticles.

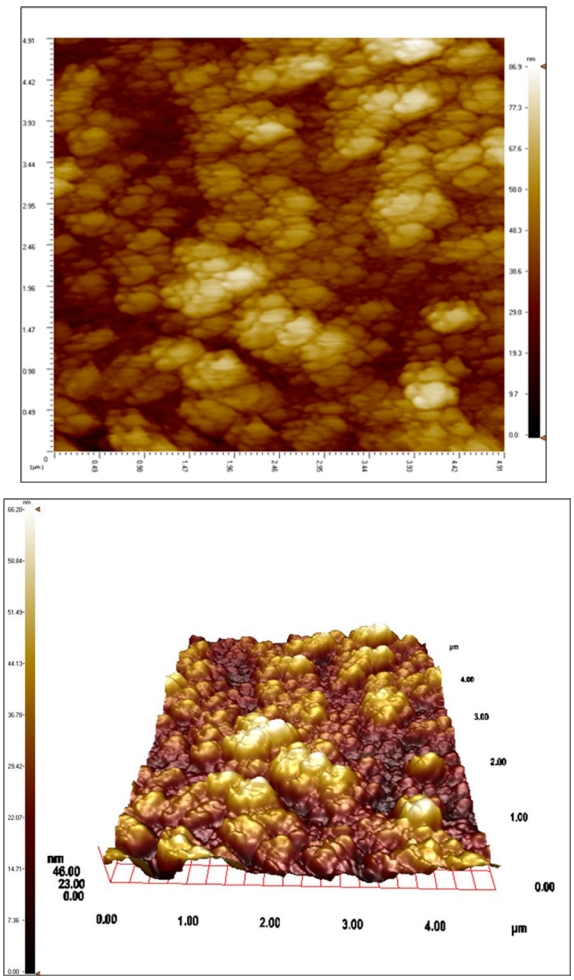

Figure 2. AFM micrograph of chitosan nanoparticles:
A) 5x5 µm surface area scan,
B) 3 D visualisation of chitosan nanoparticles.

AFM results confirm the size and dispersity data obtained from the zeta-sizer.

In the physicochemical characterization studies FTIR results have shown that ketorolac loading was successful when compared the spectrums of chitosan nanoparticles and ketorolac loaded chitosan nanoparticles with each other as given in Figure 3. Here, there are some minor shifts (especially around 1470 cm^{-1}) due to the weak forces such as hydrogen bonds, van der Waals forces and dipole moments between ketorolac and chitosan nanoparticles. Additionally typical peak at around 1425 cm^{-1} which is so clear in the case of ketorolac and ketorolac loaded chitosan nanoparticles while it's absence in the case of chitosan nanoparticles without ketorolac. Similar observations were followed in related studies [16, 17].

Drug Loading and Release Studies

In this part of the studies first of all ketorolac loading efficiency was calculated by using Equation 1 and these values were obtained as 34, 36 and 41 % for 0.8, 1.6 and 3.2 mg initial ketorolac amount sequentially. On the other hand, drug loading efficiency was changed with the changing of initial drug amount as other similar studies [18].

In release experiments; different amount of

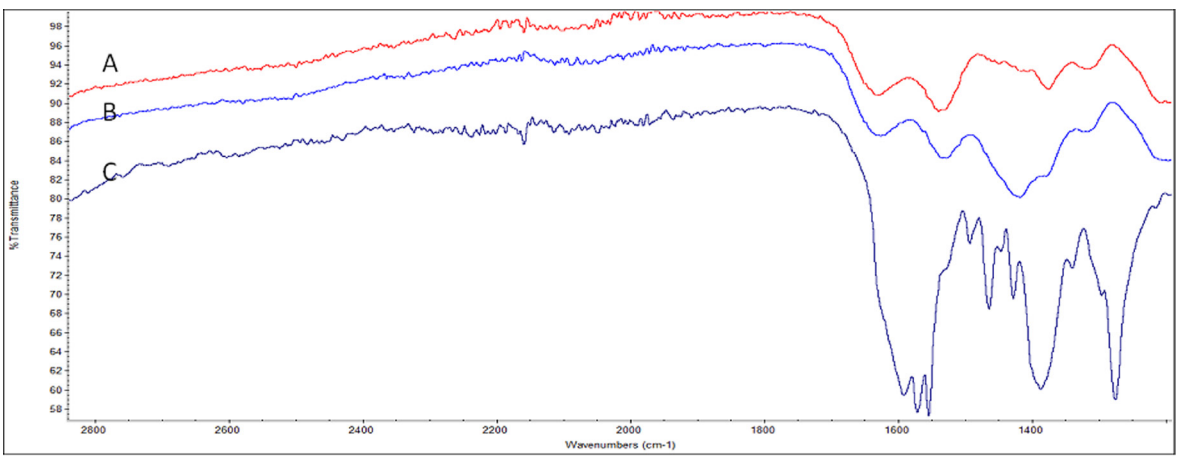

Figure 3. FTIR spectrums of chitosan nanoparticles (A), ketorolac loaded nanoparticles (B) and free ketorolac spectrum (C).

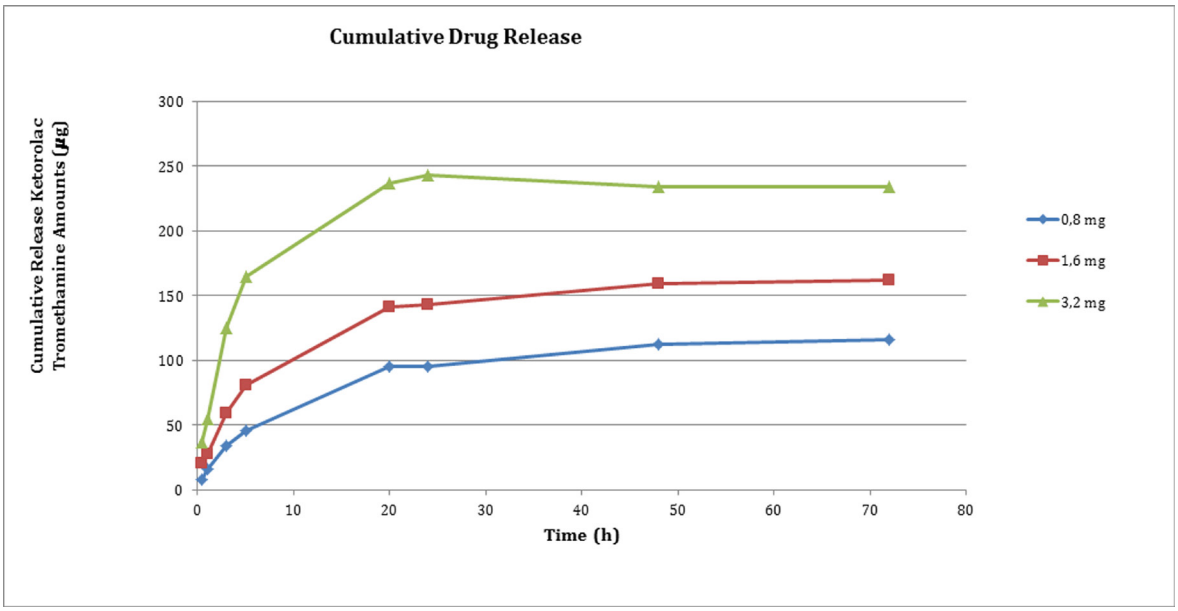

Figure 4. Cumulative ketorolac release profile of chitosan nanoparticles.

ketorolac was loaded into the nanoparticles as expressed in Experimental Section. Obtained results were given in Figure 4. The release studies were shown that an initial burst release followed by rather slower release rate were observed for a long time (i.e., up to 72 h). On the other hand, when the drug content was compared with each other for different formulations based on the release profiles, the highest drug content was caused the most rapid release rate and the rate was decreased by decreasing the drug content as expected [19].

CONCLUSIONS

Periocular injections of drugs such as corticosteroids have been used commonly for ocular inflammatory conditions and after ocular surgery. However, the side effects of intraocular steroids are well known, and the risk-benefit ratio precludes their use in some patients as mentioned before. Therefore, definitely alternatives are needed. Perhaps ketorolac loaded chitosan nanoparticles could be an alternative to treat ocular diseases such as pseudophakic cystoid macular edema according to the obtained data. Especially drug loading-release studies are very promising for the mentioned progress.

Further biocompatibility tests and in vivo studies are planned to demonstrate the suitability of this controlled release system as a novel drug carrier system for the targeted ocular diseases.

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