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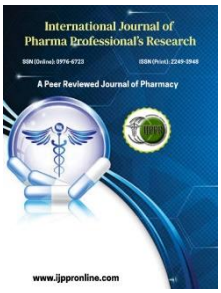


# Fabrication and evaluation of pH-sensitive nanoparticulate in-situ gel for ocular inflammation

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**ABSTRACT:** The present study was aimed to prepare and characterise pH-triggered *in-situ* gel based ophthalmic drug delivery system of non-steroidal anti-inflammatory drug (NSAID), Ketorolac tromethamine (KT). KT loaded nanocarriers were developed by ionotropic gelation technique using chitosan as a matrix forming polymer, cross-linked by an anionic crosslinker sodium tripolyphosphate (TPP). Benzalkonium chlorides at suitable concentration were used as a preservative. The formulations were sterilized by moist heat sterilization as per I.P. The prepared nanoparticles were converted into in-situ gel using Gallen gum. The prepared formulations were evaluated for clarity, pH measurement, gelling capacity and *in-vitro* diffusion study. Under rheological investigation both solution and gel were found to be in pseudo plastic behaviour. The selected formulations showed sustained release over a period of more than 12 hrs with increased resident time. All studies shown favourable results; thus, *in-situ* gelling system is a valuable alternative to counter precorneal loss a major drawback in the ophthalmic preparation.

## 1. Introduction

The eye is an interesting organ. The tear flow and blinking reflex maintain a good environment and remove foreign material from the eye. Inflammation is the indication of cellular and vascular response of the host tissue to injury which may be inflicted by physical or chemical agents, invasion of pathogens, ischemia, and excessive (hypersensitivity) or inappropriate (autoimmunity) operation of immune mechanisms. Post-operative inflammation includes distracted aqueous-blood barrier, excessive blood flow in conjunctival vessels, miosis, elevated IOP, intervened by COX pathways.[1-2] Ocular inflammation most frequently hastens due to infection, allergens, surgical intervention or trauma.[3] Various ocular problems/diseases i.e. intra¬operative miosis (during cataract surgery), postoperative inflammation, cystoid macular edema (CME) following cataract extraction, seasonal allergic conjunctivitis, ocular dis¬comfort (pain and

photophobia) after refractive sur¬gery, Diabetic retinopathy (DR) and Diabetic macular edema (DME) are prevented/treated by various drugs belonging to NSAID class.[3]

In ocular drug delivery, the physiological constraints imposed by protective mechanism of the eye lead to low absorption of drugs and sometimes short duration of therapeutics effect. One of the reasons for relatively low bioavailability of conventional eye drops is their short precorneal contact time. When drug solution is administered in the form of drops, effective tear drainage and blinking results in a 10-fold decrease in drug concentration in 4 to 20 min.[4] The drug absorption is also dependent upon the chemical nature of the drugs since the corneal permeability depends upon molecular size and hydrophobicity of drugs.[5] By tear drainage the main part of administered drug is transported via the naso-lachrymal duct to the gastrointestinal tract where it may be absorbed, sometimes causing the systemic side-effects. Rapid elimination of administered eye drops often results in a short duration of therapeutic effect making a frequent dosing regimen. In order to increase the effectiveness of the drug, a dosage form should be selected, which increases the contact time of the drug in the eye. This may increase bioavailability, reduce systemic absorption, and reduce the need for frequent administration leading to improved patient compliance. Ocular therapy would be significantly improved if precorneal residence time is increased and the most common way to achieve this is by increasing the viscosity of the solution. Gels and ointments moderately affect the contact time of the drug and have long residence time. They have a low patient compliance as they blur the vision and are recommended for bedtime use.[6-7]

Ketorolac tromethamine is a nonsteroidal anti-inflammatory drug, used to treat seasonal allergic conjunctivitis. At present, it is available in the form of eye drops, which need to be administered 1 or 2 drops every 15 to 30 min. initially in acute infection and 1 or 2 drops administered 4 times daily or more in severe conditions. To overcome these limitations associated with dosage regimen, an attempt has been made to formulate nanoparticulate in-situ gelling system that may not only improve the efficiency of the therapy but also patient compliance. Several polymeric systems have been used to fabricate in-situ gels for better ocular bioavailability and retention to drug of which gelling systems have shown advantages of convenient administration and increased contact time. The purpose of this study was to develop a nanoparticles laden in-situ gel of Ketorolac tromethamine using polymeric system of TPP as gelling and chitosan as bioadhesive agent. The prepared dosage regimens provided ease in the application and capable to sustained drug release with reduced frequency of administration.[8-11]

## Material and Methods

### Material

Ketorolac tromethamine was obtained as gift sample from Panchsheel Organics Ltd, Indore. Other ingredients like sodium tripolyphosphate, chitosan and other chemicals were procured from various reputed companies.

### Methods

#### Drug Excipient compatibility study

Drug (Ketorolac tromethamine)-excipient compatibility was checked by adopting IR spectroscopy. Infra-red spectra of pure drug alone and with chosen ingredients were recorded on FTIR (Shimadzu IR Affinity-1) spectroscopy at a resolution of 2 $cm^{-1}$ ranged from 4000 to 400 cm-1 using KBr discs.

#### Preparation of Ketorolac tromethamine loaded Chitosan nanoparticles

Drug (Ketorolac tromethamine) laden nanoparticles were produced by ionotropic gelation method using chitosan and sodium tri-

polyphosphate (Na-TPP). Prepared product (suspension) was centrifuged (Remi, Mumbai, India) for 15 min. at 12000 rpm. Sediment was freeze dried (Lyophilizer, Biogen-model no BGS214, India) for 48 h. The obtained nanoparticles were stored under dehydrated conditions for further studies.[3, 12]

**Experimental design**

Design Expert (Version 13, Stat-Ease Inc., Minneapolis, MN) was used for optimization study of Ketorolac tromethamine nanoparticles consisted of 2 independent factors i.e. amount of chitosan (X1) and sodium tri-poly phosphate (X2) which were evaluated, at 3 levels (-1, 0, 1 i.e. 100, 200 and 300 respectively) and 9 experimental trials were performed with reference to particle size and percent (%) entrapment as dependent variables. All other processing variables were kept constant throughout the study.

**Physicochemical characterization of Ketorolac tromethamine nanoparticles**

The average diameter and zeta potential of drug-loaded nanoparticles were measured by dynamic light scattering method using Zetasizer nano zs (Malvern instrument ltd., Worcestershire, UK). Surface morphology was used to determine the shapes and distribution of particles and measured by SEM (XL series Quanta FEI 200F).

***In-vitro* Drug release**

Drug release from drug-loaded nanoparticles was carried out by suspending the prepared nanoparticles in PBS (phosphate buffer saline, pH 7.4) in a conical flask (100 ml) and shaked on magnetic stirrer (150 cycles/min) at 37°C. Withdrawn samples were centrifuged at 12000 rpm and analyzed using UV spectrophotometry at 322 nm.

**Preparation of Nano particulate in-situ gel of Ketorolac tromethamine**

Nano particulate *in-situ* gel of Ofloxacin was prepared by using gellan gum because it showed highest viscosity that persists for longer duration of time and thus selected for further work. Gellan gum was dispersed in deionized water, heated to 90°C while stirring and then cooled to room temperature. Later on, drug loaded nanoparticles (equivalent to the prescribed dose of the drug) were dissolved in purified water and allowed with the addition of benzalkonium chloride. Then this solution was added to the above solution. The pH was adjusted in the range of 5.0-6.0 using 0.1 M NaOH Solution. The volume was made-up to 100ml with purified water. Prepared formulations were sterilized in an autoclave at $121^0$C and 15psi for 20 min.[13]

**Evaluation of Ketorolac tromethamine nanoparticulate in-situ gel**

The prepared nanoparticulate in-situ gel of Ketorolac tromethamine was evaluated for clarity, pH, gelling strength, viscosity and in-vitro diffusion study.

**Accelerated (Physical) Stability studies**

ICH (International Conference of Harmonization) guidelines were followed to carry out the stability study of optimized formulation. The optimized formulation was stored at accelerated conditions (400±20C/75%±5% RH) for six months respectively and reckoned, for released characteristics, at predetermined time period.[14]

**Results and Discussion**

**Drug Excipient compatibility study**

The FTIR spectra of pure drug resulted in characteristic peaks which resembled with those obtained with the combinations. In the spectrum of ketorolac tromethamine (Figure 2A), major peaks (3,625 cm-1; NH stretch); 1,157 cm-1; C = O stretch (diaryl ketone); 1,625 cm-1; C-C stretch (aromatic stretching); and 3,650 cm-1; OH (acid); 2679 cm-1; CH stretching vibration,

1172 cm-1; C–O–C and 1908 cm-1; NH2, were observed in spectra obtained with chitosan.

**Formulation, Optimization and Evaluation**

In the present investigation, selected independent variables were amount of chitosan and TPP while the particle size and percentage drug entrapment was selected as dependent variables. The selected independent variables were studied at different levels to observe their individual as well as interactive effects.

Particle size of prepared nanoparticles ranged between 159.2- 225.5 nm and quadratic model was best suited for the data which was validated by ANOVA. The factor A had shown more effect on Particle size of nanoparticles ($p$<0.0001) than factor B ($p$<0.05). The predicted $R^2$ of 0.9898 was significantly correlated with adjusted $R^2$ of 0.9977; i.e. the difference is less than 0.2. Signal to noise ratio greater than 4 is desirable, and in the present study, it is 75.719 that indicated an adequate signal.

The particle size decreased significantly with increase in concentrations of both factors i.e. chitosan and TPP which was presented in the form of contour and 3D response surface plots. It is already reported that as the concentration of chitosan and TPP increased, particle size decreased accordingly.

Second dependent factor was chosen as percent (%) drug entrapment. Entrapment efficiency of prepared batches (F1-F9) was found between 55.0%-79.21% (Figure 1). Both factors A and B had similar effect on entrapment efficiency of nanoparticles ($p$<0.0001). The predicted $R^2$ of 0.9998 was in correlation with adjusted $R^2$ of 0.9999; i.e. the difference is less than 0.2. Signal to noise ratio was 466.0; indicated an adequate signal.

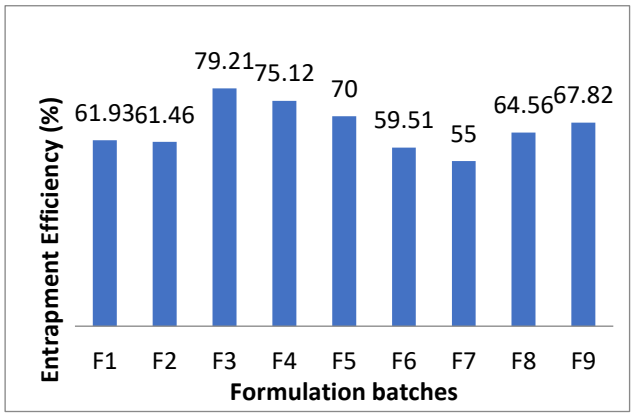


**Figure 1:** Entrapment efficiency (%) of Ketorolac tromethamine nanoparticles formulation batches (F1-F9)

From the results, it was shown that when factors i.e. A and B increased, the drug entrapment into polymer was also increased. It is reported that a decrease in weight ratio of CS/TPP, decreases the encapsulation efficiency of nanoparticles pertaining to more compact solid-matrix structure that had led to the increasing amount of nanoparticles formation, resulting in the increased nanoencapsulation. Moreover, the maximum polymer and surfactant (TPP) concentration give more encapsulation efficiency and vice-versa. It may be due to fact that increased size of polymer can encapsulate more drug, but more surfactant also accelerate the encapsulation by increasing the binding contact between drug and polymer in emulsion stage. Desirability function was used for optimization to obtain the levels of process parameters in which particle size was kept at minimum and entrapment efficiency at maximum. Based on the results obtained from predicted solution given by optimization study, a checkpoint batch (KTopt) was prepared and considered as optimized batch for further studies.

The Scanning Electron Microscope (SEM) is most versatile instrument available for examination and analysis of morphology and chemical characterization of nanomaterials. It is an important tool for characterization of particle morphology and its distribution. Ketorolac

tromethamine nanoparticles (batches F1-F9) were having rough surfaces with spherical in shape.

The release rate decreased with increase in cross-linking density. A dense matrix of the nanoparticles might exhibit slower release rate of the drug. Percentage drug release from Ketorolac tromethamine nanoparticles was found to be in 89.32-95.66% and 87.34-93.15% in pH 7.4 PBS and Simulated Tear Fluid (STF), respectively.

The optimized batch thus prepared was having % Entrapment Efficiency (79.53%), Particle size (155.8 nm), Zeta Potential (-22.8 mV) and percentage drug release (89.49±0.55%) in STF (pH 7.4). The % drug release data was presented in figure 2. Results of release kinetic study revealed that drug dissolution followed Zero order release kinetics model. The nanoparticles were found stable under accelerated stress conditions.

Nano particulate *in-situ* gel of Ketorolac tromethamine thus prepared was evaluated for various parameters i.e. clarity, pH, viscosity, gelling strength and *in-vitro* diffusion study. Under rheological investigation both solution and gel was found to be in pseudo plastic behaviour. The *in-vitro* drug release from Nano particulate *in-situ* ocular gel was found to be 82.51±1.18% and 81.72±1.25% in pH 7.4 phosphate buffer and simulated tear fluid (STF), respectively, over a period of 12 h.

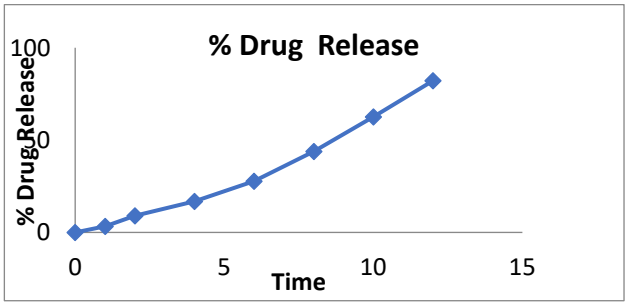


**Figure 2:** Percentage (%) drug release from Ketorolac tromethamine nanoparticles (Optimized batch, KTopt) at the end of 12 hr.

## Conclusion

In the present study, Nano particulate *in-situ* gel of KT was prepared for the treatment of ocular inflammation by utilizing the previously prepared nanoparticles. Initially, nanoparticles of Ketorolac tromethamine were prepared and evaluated for various evaluation parameters. Full factorial design was adopted to optimize the formulation and based on the predicted solution given by the software a checkpoint batch was selected and considered as an optimized batch. Nano particulate *in-situ* gel was prepared with the optimized batch and prepared gel was further evaluated for various parameters and satisfactory results were obtained. Hence, it was concluded that the research work, in reference, comprised of quite novel approaches of investigations.

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