Study on Topical Ocular Delivery System of Certain Non-steroidal Anti-inflammatory Drugs

Thesis Presented by

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ABSTRACT

Aqueous ocular drops of ketorolac tromethamine (KT) are effective and safe for topical use in inhibiting postoperative inflammation of the eyes, reducing postoperative pain, reducing conjunctivitis with no alteration of corneal opacity, and do not increase intraocular pressure. Also, it can be considered as a viable alternative to corticosteroids in treatment of ocular inflammation in the presence of pathogens.

Eye drops are favored due to cost advantage, greater simplicity of formulation development and production and the good acceptance by patients despite a little blurring. However, these conventional systems cannot be considered optimal in the treatment of vision-threatening ocular diseases as most of the drugs are washed from the eye and only less than 5% of the administered drugs could penetrate the cornea to reach the desired intraocular tissue due to various mechanisms as lacrimation, tear dilution, and tear turnover. Also, the high frequency of eye drop instillation is associated with patient non-compliance.

Polymeric nanoparticles are ranged in size between 10-1000 nm. They are prepared from biocompatible and biodegradable polymers where the drug may be dissolved and entrapped or encapsulated or may be attached to a nanoparticles matrix to achieve controlled drug delivery. Several polymers such as chitosan, alginate and Eudragit have been widely used in pharmaceutical industry for nanoparticles synthesis. Cationic polymers probably have superior mucoadhesiveness due to their ability to develop molecular attraction forces by electrostatic interactions with the negative charges of the mucus.

The work in this thesis is divided into four chapters:

Chapter I: Preparation and Evaluation of Ketorolac Tromethamine-Loaded Nanodispersions by Different Techniques

In this chapter, ketorolac tromethamine was formulated in different types of nanodispersions using different cationic polymers (chitosan, sodium alginate and Eudragit RL100) that were prepared with different techniques. Cationic polymers were selected to enhance the mucoadhesion of nanoparticles by electrostatic interaction with the negatively charged mucin of the cornea and conjunctiva.

1. Chitosan/ tripolyphosphate (CS/TPP) nanoparticles

Prepared by ionic gelation method according to the procedure first reported by Calvo *et al.* Different amounts of CS were dissolved in 1% acetic acid, adjusted to pH 5.5 with NaOH, to give concentrations of (0.3, 0.45, 0.6, 0.75 mg/ml). Under magnetic stirring at room temperature, 4 ml of TPP aqueous solution with various concentrations (0.2, 0.4, 0.6, 0.8, 1 and 1.2 mg/ml) were added into 10 ml of CS solution. Three kinds of phenomena were observed: solution, aggregate and opalescent dispersion. The formulations which showed opalescent dispersion were considered as suitable plain CS/TPP nanoparticles for incorporation of ketorolac tromethamine (KT).

2. Alginate/ chitosan (ALG/CS) nanoparticles

Prepared by two different techniques; modified coaservation and ionotropic pregelation method. Both of the sodium alginate and chitosan solutions were prepared by dissolving the polymers in distilled water and 1% acetic acid, respectively. The pH of the sodium alginate solutions (5.0–5.3) was adjusted using hydrochloric acid; the pH of CS solution was modified to 5.5 with NaOH.

a) ***Modified coaservation method***

According to Calvo *et al*. method, 4 ml of the aqueous solution of sodium alginate in different concentrations (0.2, 0.4 and 0.6%) were sprayed into 10 ml of different concentration of chitosan solution (0.1, 0.2, 0.3 and 0.45%) under continuous magnetic stirring at 1000 rpm for 30 min. Nanoparticles were formed as a result of the interaction between the negative groups of ALG and the positively charged amino groups of CS (ionotropic gelation).

b) Ionotropic pregelation method

It is a two steps method. The first step in the nanoparticles preparation is the formation of calcium alginate pre-gel by adding 6 ml of aqueous calcium chloride solution to 10 ml sodium alginate solution (pH 5.4) while stirring at 400 rpm for 30 minutes. The second step was the addition of 4 ml of chitosan solution (pH 5.2) to the resultant calcium alginate pre-gel with continuous stirring for another 30 minutes. The resultant colloidal dispersion was equilibrated overnight at room temperature to allow nanoparticles to form uniform particles. The required amount of KT was dissolved in the sodium alginate solution to give the desired concentration (0.5% w/v).

The effect of individual experimental parameters (calcium chloride, sodium alginate and chitosan concentrations) on the particle size, zeta potential, entrapment efficiency and *in-vitro* release of the nanoparticles was studied while all other variables (stirring times, volume of calcium chloride solution) were kept constant.

c) Eudragit RL100 nanoparticles

Prepared by solvent displacement technique (nanopercipitation method) using different ratios of KT: Eudragit RL100 (1:1, 1:3 and 1:5) but constant weight of KT (100 mg). Eudragit RL 100 and KT were first dissolved by sonication in specified volume of anhydrous ethanol or, acetone /methanol mixture (3:1). This organic phase was slowly poured with constant speed (0.5 ml/min) into calculated volume of aqueous phase (distilled water or citrate-phosphate buffer of pH 3) containing 1% polyvinyl alcohol (PVA), as hydrophilic surfactant, under moderate magnetic stirring at 800 rpm, where the nanoparticles were formed spontaneously.

In order to obtain nanoparticles with suitable size, zeta potential, entrapment efficiency and *in-vitro* release, the process conditions and formulation factors were investigated. The tested parameters were: type of aqueous phase, ratio of drug to polymer, type of organic phase and organic to aqueous phase ratio. Only one parameter was adjusted in each series of experiments while all other parameters were kept constant.

<u>In CS/TPP NPs</u>

All the plain formulations showed positively charged zeta potential and the size of all formulations ranged from 111.1 nm to 613 nm, except C16 which exceeded the size of 1000 nm, and was thus excluded from the formulations used for drug loading. The size and zeta potential of CS/TPP nanoparticles increased by the addition of KT, and the entrapment efficiency of KT-loaded CS/TPP NPs formulations ranged from 40.11% to 57.99%. Increasing the concentration of TPP caused a significant increase in particle size due to accumulation of excess TPP on the surface of the particles, but associated with a significant decrease in zeta

potential. However, increasing the concentration of CS caused a significant decrease in the particle size but the zeta potential significantly increased. The particle size of C9 significantly increased from 147.2 nm to 565.1 nm as the concentration of KT increased from 0.3 mg/ml to 5 mg/ml. However, the zeta potential and entrapment significantly decreased.

In ALG/CS NPs

Following modified coacervation method, not all ALG/CS ratios resulted into the formation of colloidal dispersions but all the formulations prepared by ionotropic pregelation method were nanodispersions with smaller particle size.

In modified coaservation method, increasing either CS or ALG percentage resulted in a significant increase in particle size. However, this effect was not observed in ionotropic pregelation method. When ALG exceeded CS, the formulations showed negatively charged zeta potential in modified coaservation method but in ionotropic pregelation, Ca^{2+} ions also play a role in the overall charge on the particles. The particles showed positively charged zeta potential only at high concentration of chitosan and alginate.

There was non-significant difference between the EF % of the different formulae prepared by the same method but it was lower in nanoparticles that were prepared by ionotropic pregelation method due to the presence of $CaCl_2$ which made the core of the particles.

In Eudragit nanoparticles

Using the citrate-phosphate buffer (pH 3) as an external aqueous phase showed a significant increase in the entrapment efficiency of KT to 91.61% than using distilled water because KT is highly soluble in water and when the organic

phase was added, part of KT was ionized and was escaped from the nanoparticles but KT (pKa 3.5) is insoluble in citrate-phosphate buffer pH 3.

Particle size and zeta potential significantly increased as the polymer content increased proportionally due to the cationic nature of Eudragit RL100, but the entrapment efficiency decreased due to formation of more compact polymer coat which hindered the entrapment.

Increasing the volume of the external aqueous phase, having 1% polyvinyl alcohol, as a surfactant, resulted in a significant decrease in the particle size because the presence of the particles in large volume decrease the chance of aggregation, and also the entrapment of KT decreased but the value of zeta potential increased.

The formulation containing ethanol as organic phase showed smaller particle size, lower zeta potential and higher entrapment efficiency when compared to that prepared with acetaone/methanol (3:1) due to higher polarity and faster evaporation of ethanol.

All NPs had sustained release profiles when compared with Acular® eye drops (aqueous solution). CS and ALG nanodispersions showed an initial burst release followed by a more gradual sustained release. However, the Eudragit NPs showed prolonged release without burst phase due to the higher entrapment efficiency and lower amount of free KT in Eudragit nanodispersions.

C9b (CS/TPP), A18 (ALG/CS) and E2 (Eudragit RL100) nanodispersions were selected for further studies (morphology, IR-spectroscopy and physical stability study) as they have small particle size, high positive zeta potential and maximum entrapment efficiency.

The morphology of the nanoparticles was examined by transmission electron microscope; it was found that CS/TPP nanoparticles were of irregular shape while the ALG and Eudragit NPs were destincit, spherical with smooth surface. Furthermore, by TEM the size of CS and ALG nanoparticles was smaller than the size of Eudragit NPs but the opposite was obtained by zetasizer, this may be attributed to that the zetasizer measures the apparent size (hydrodynamic radius) of a particle, including hydrodynamic layers that form around hydrophilic particles such as those composed of CS and ALG, leading to an overestimation of NPs size.

The FT-IR analysis of the selected KT-loaded nanoparticles that were freeze dried was performed to confirm the successful entrapment of KT in the nanoparticulate systems.

The selected samples were stored at different temperatures (25°C and 4°C) for six months away from light and they were evaluated at specified time intervals (1, 3 and 6 months) for visual examination, particle size, pH and entrapment efficiency. All the NP dispersions showed increase in particle size due to aggregation, but decreasing in pH values and also entrapment efficiency was decreased with time due to leakage of drug. All nanodispersions were more stable at 4°C than at 25°C and the Eudragit nanodispersion was the most stable one.

Chapter II: Preparation and Evaluation of Polymeric Films Containing Ketorolac Tromethamine Nanodispersions

In this chapter, mucoadhesive polymeric films containing nanodispersions were developed as a novel ocular drug delivery system to maintain a therapeutic level at the site of action for prolonged period of time, thus, decreasing the frequency of administration and increase the patient compliance, and to overcome

the disadvantage of nanodispersions in being thermodynamically unstable at room temperature.

Two polymeric films were prepared in this study; one of them consisting of 10% hydroxyl propyl methyl cellulose (hydrophilic polymer) and 10% glycerin as plasticizer, the other system consisting of 2.5% Eudragit RL100 (hydrophobic polymer), 1.25% hydroxyl propyl methyl cellulose and 30% polyethylene glycol 400 (plasticizer).

The polymeric films of KT-loaded nanodispersions were prepared by solvent-cast technique and then were evaluated.

The thickness of different films was measured by a screw gauge. The folding endurance was determined to give indication about brittleness. The weight uniformity, percentage of moisture loss and absorption were determined using digital balance. The swelling index was determined gravimetrically using agar gel plate. Mucoadhesive strength of the films was measured with a modified balance and the force of adhesion was calculated. Mechanical properties of the films (elongation at break and tensile strength) were determined.

It was found that, the films prepared with Eudragit/HPMC K4m/PEG400 showed more flexibility and elasticity due to high concentration of PEG 400, less thickness and weight due to lower concentration of polymers, higher folding endurance, lower percentage of moisture loss due to hydrophobicity of Eudragit which hindered the transfer of water, lower percentage of moisture absorption, higher mucoadhesive strength due to the electrostatic attraction between the cationic Eudragit and the anionic mucin and due to the mucoadhesive properties of PEG 400 and lower swelling index due to poor wetting properties of the hydrophobic polymer, Eudragit.

In vitro drug release study was carried out by using USP dissolution apparatus that was adjusted at 35ºC and 50 rpm with PBS as dissolution medium. Incorporation of the KT-loaded NP dispersions in polymeric films showed significantly lower percentage of KT released when compared with their NP dispersions which was less considerable in Eudragit nanodispersionsdue to lower amount of free KT in Eudragit nanodispersion. The presence of Eudragit RL100 in combination of HPMC K4m, in (NF4 - NF6) films, caused a significantly lower percentage of KT released than HPMC E15 alone due to poor water solubility and hydrophobicity of Eudragit.

The best fit kinetic model for all formulations (NF1-NF6), except NF5 (Korsemeyer-Peppas), was the Higuchi diffusion kinetic model. The diffusion of water soluble drug through the gel layer has been shown to be linearly dependent on the square root of time because the drug release from such systems usually involves the uptake of water by the polymer and subsequent swelling to form a gel layer, which controls drug release by viscous resistance to drug diffusion. However, the swellable polymer systems also exhibit erosion, which introduces another release mechanism. So that, the n-values were between 0.5 and 1 which indicates non-Fickian (anomalous) transport mechanism, except NF5 (n=0.37), which means that the drug is released from these films via diffusion mechanism and also another process called chain relaxation, but in NF5 it was controlled by diffusion only.

Physical stability studies were carried out on the most satisfactory formulation (NF6) that was prepared with Eudragit nanodispersion in Eudragit/HPMC K4m/PEG 400. It was stored in aluminium foil for six months and its physicochemical properties (thickness, weight and folding endurance) and the total percentages released were evaluated at different time intervals and it was found to have good physical stability.

Chapter III: Preparation and Evaluation of *In-Situ* Gels Containing Ketorolac Tromethamine Nanodispersions

In-situ gel systems combines the advantages of both gels and solutions in having longer residence time and ease of administration, respectively. They exhibit sol-gel phase transition due to change in specific physicochemical properties (e.g. pH, temperature and ions)

In this chapter, mucoadhesive temperature sensitive *in-situ* gels containing nanodispersions were developed as a novel ocular drug delivery system to prolong the pre-corneal drug contact time for enhancing diffusion and to improve ocular bioavailability. As well as from the point of view of patient compliance, a liquid dosage form that can sustain drug release and remain in contact with the cornea for extended periods of time is ideal. This can be achieved by using a polymer (e.g. Pluronic F-127) that occurs as a solution at room temperature (below 25°C) and convert to gel at precorneal temperature (34-35°C) after dilution with tear fluid.

Aqueous solutions of Pluronic F-127 and HPMC K4m with different concentrations were prepared, by cold method, and evaluated for gelling capacity and transparency. The in situ gel bases that showed immediate gelation that was remained for few hours were selected.

The KT-loaded nanodispersions were added to the *in-situ* gels (20% PF-127 and 14% PF-127+1.5% HPMC) that showed optimum gelling capacity, and then they were evaluated for gelation time, gelling temperature, pH, rheological properties and in vitro release to select the optimum formulation.

The general appearance of all formulae was homogenous and colorless but translucent, and they had pH values of 5-7.

The time required for the polymeric system to transform from sol to gel at its gelation temperature was determined and was from 23 to 43 seconds. Also, the gelation temperature was observed in range of 29-34°C, and it decreased as the concentration of PF-127 was increased due to increasing the critical micelle concentration.

By determining the mucoadhesive strength, the results revealed that the addition of HPMC K4m to Pluronic formulations improved their mucoadhesion due to its wetting and swelling properties.

The viscosity and rheological behavior of *in-situ* gels were evaluated by Brookfield viscometer. By comparing the apparent viscosities, it was found that the viscosity increased with increasing the temperature and the formulations changed from sol state, at 4ºC, to the gel state, at 34ºC. Also, it increased by increasing the concentration of PF-127.

All the *in-situ* gel formulations loaded with nanodispersions exhibited shear thinning flow since the viscosity decreased with increasing the shear rate, and the values of "N" that indicate the deviation from the Newtonian flow were greater than one indicating pseudoplastic flow and showed thixotropic properties.

The *in-vitro* release of KT was performed to study the effect of incorporation of nanodispersions in the *in-situ* gels which was observed to eliminate the burst release in both of CS and ALG nanodispersions and also decreased the percentage of KT released, but decreasing the concentration of PF-127 showed non-significant increase in release of KT due to the presence of HPMC K4m with the lower concentration of PF-127 (14%).

It was found that KT release from the formulations prepared with PF-127 alone (NG1-NG3) followed Korsemeyer-Peppas model. However, the formulations prepared with PF127 /HPMC K4m (NG4-NG6) followed first-order kinetics (concentration dependant) since a straight line was obtained when log %

cumulative drug retained versus time was plotted. Also, the same result of first order release kinetic mechanism was obtained from *in-situ* gels containing clotrimazole for oral candidiasis.

All of the *in-situ* gel formulations (NG1-NG6) had *n*-values in the range between 0.5 and 1 ($0.5< n <1$) suggesting the non-Fickian (anomalous) release mechanism for the drug "*i.e.*" both erosion and diffusion.

Physical stability studies were carried out on the most satisfactory formulation (NG6). There was no significant change in the physicochemical properties (clarity, color and pH), the viscosity was non-significantly decreased from 324 to 298 cps while the total percentage of KT released after 6 hours increased from 15.01 to 18.83% after six months which may be attributed to the decrease in viscosity by time.

Chapter IV: *Ex-vivo*/*In-vivo* Transcorneal Permeation and Irritation Studies on the Optimum Preparations of Ketorolac Tromethamine

In this chapter, the *ex-vivo* transcorneal permeation study was performed on the selected formulae from previous chapters (C9b, A18 and E2) that were named as F#II, F#III and F#IV, respectively, and they were compared with Acular® eye drops (F#I) which had the same concentration of KT (5mg/ml).

The *ex-vivo* transcorneal permeation was carried out as previously described in release study using freshly excised bovine corneas as membrane at 35°C± 0.5 and at 50 rpm in PBS pH 7.4. The cumulative amount of KT permeated after 1.5 hours was determined then the permeation parameters (flux and permeability coefficient) was calculated.

The permeation pattern of the studied formulae was in the following order; F#II (nanodispersion) > F#III (in situ gel) > F#IV (film) > F#I (eye drops).

Nanodispersion showed the highest permeation due to the good uptake of cationic and small size nanoparticles, followed by insitu gel and film due to the inclusion of free part of KT in polymeric network which was hydrophilic network in the in-situ gel but hydrophobic in the film. The aqueous eye drops showed the lowest permeation due to the high molecular weight of KT which is hydrophilic in nature that hindered its permeation through both of paracellular and transcelluar transport.

Corneal hydration was determined at the end of the permeation experminent. Each cornea was weighed, soaked in one ml methanol, dried overnight and reweighed. All the formulations showed corneal hydration level within the acceptable range (75-80%) indication no corneal damage.

The *in vivo* study was performed using 8 albino rabbits that were divided into four groups; each group had two rabbits and four eyes. The concentration of KT in aqueous humor was determined by HPLC and was compared.

The maximum concentration of KT after Acular eye drops was after one hour and no KT was detected in the aqueous humor after 6 hours, but the maximum concentration from nanocarrier formulations was after two hours, and KT still detected in aqueous humor for more than eight hours which indicated their ability in prolonging the effect of drug due to increasing the precorneal residence time.

The pharmacokinetic parameters were determined and were compared with aqueous eye drop (Acular®). KT-loaded Eudragit nanodispersion and nanodispersion/in situ gel formulations provided higher C_{max} and AUC_{0-8}. All the formulations showed longer T_{max} and $t_{1/2}$ than the Acular® eye drops and exhibited prolonged effect.

In situ gel formulation (F#III) showed the highest ocular relative bioavailability in the aqueous humor which was about 250% followed by the nanodispersion (about 203%). However, the film showed the lowest bioavailability (about 95%).

According to the pharmacokinetic parameters, the in situ gel incorporated with nanodispersion (F#III) was selected as an optimum formulation for further study of irritation using Draize test and winking test.

In Draize test, three albino rabbits were used as the control eyes received no sample and the test eyes received 50 µl of F#III. The irritancy was tested for redness, swelling and watering of the eye at the time interval of 1, 24, 48 and 72 h.

All of them showed grade zero which indicated non irritant effect on eyes.

In winking test the frequency of the winking of the rabbits, for 5 minutes, was recorded and compared with the result of control eyes that received normal saline solution. No irritation effect was observed for F#III.

General conclusion

Eudragit nanodispersion incorporated into 14% Pluronic/1.5% HPMC K4m insitu gel can be considered as potential ophthalmic dosage form for preparation of Ketorolac tromethamine for treatment of post operative ocular inflammation especially after cataract surgeries and can improve the patient compliance and decrease the probability of wound infection due to less frequency of administration as it showed prolonged effect.