**Intravitreal Implantable Film Containing Ketorolac Tromethamine Microsponges – In Vitro/In Vivo Correlation**

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### ABSTRACT

This research involved preparation, optimization, in vitro and in vivo evaluation of Ketorolac Tromethamine (KT) from the implantable film containing KT-microsponges to be inserted at the posterior part of the eye and may treat some complicated conditions such as cystoid macular edema (CME) and diabetic macular edema (DME). Specific amount of our previously prepared KT microsponges (equivalent to 6 mg KT) was added to a film base composed of poly vinyl alcohol (PVA) and glycerol to which 3 mg uncoated (pure) KT added then casting the combination to get film (0.6 cm x 0.3 cm). The prepared film was evaluated for weight uniformity, thickness, folding endurance, surface pH and the percentage of moisture absorption (PMA) and percentage moisture loss (PML) as well as in vitro release and in vivo tests. The obtained film showed average weight (8.87±mg ± 0.2) with variation less than (5%), thickness (0.3 cm ± 0.04), surface pH ranged (7-7.3), folding endurance more than (300) times indicating excellent elasticity with good integrity through measuring percentage moisture absorption (PMA) and percentage moisture loss (PML). The in vitro release showed biphasic release where 40% of the drug was released within the first day (initial phase) followed by sustained release continued to give 90% of the drug within 3 months. The in vivo test showed that the implanted film in the vitreous chamber of the rabbit eye had good anti-inflammatory properties against acute and chronic inflammation as well as prevent recurrent inflammation. The work succeeded to prepare KT film containing microsponges to be implanted intravitreally at the posterior chamber of the eye and suggested to treat complicated condition including postoperative cystoid macular edema as well as diabetic macular edema as alternative to repeated intravitreal KT injections, so decreasing side effects with improved patient compliance.

**Keywords:** Implant film, ketorolac tromethamine, microsponges, poly vinyl alcohol

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### INTRODUCTION

Topical drops and intracocular injections are the primary delivery routes for eye diseases. The prominence route is the topical administration as their low invasiveness and simplicity but patient compliance and insistence are the problematic issues with this route. Furthermore, the permeability of therapeutics topical drops is insufficient to achieve effective therapeutic concentration at the posterior part of the eye and it is rarely used for treating retinal diseases (1). With the introducing of NSAIDs to treat postoperative cystoid macular edema (CME) and diabetic macular edema (DME), intracocular injection be a common technique for using the drugs for retinal diseases (2). Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory drug belongs to nonselective COX inhibitor group used to control moderate to severe pain as analgesic (3) as well as intravitreal ketorolac tromethamine injection (Toradol® 30mg/ml) used as anti-inflammatory agent to treat chronic cystoid macular edema after complicated cataract surgery (4). Despite the efficacy of intracocular injection, its impact on clinicians, invasiveness and cost of therapy has led to substitutional protocol for reducing treatment frequency. Currently, intraocular implants (biodegradable polymer) overcome the drawbacks of intracocular injections as they offer stable long-term vitreous concentration (in therapeutic range) of drugs (5). Implantable film was used since the thin film is less friable, easy to carry in contrast to the conventional dosage forms (6), comparing to liquid dosage forms, it is more stable (7), in contrast to eye drop, it gives better ocular bioavailability with sustained drug delivery leading to reduction in frequent dose and systemic side effect lead to more patient compliance (8). There are many methods to prepare implantable film but the most common method is solvent casting technique as it is feasible and the most exceedingly used technique as it is straightforward process with low cost (9). Poly Lactic Acid (PLA) and Poly Lactic-co-Glycolic Acid (PLGA) are widely used to prepare implantable film due to its safety and excellent biocompatibility (10). As well many methods have been used to sustained the drug release, one of them is microsponges which is polymeric delivery system consist of spongy microspheres characterized as a tiny, inert non-collapsible structure with particle size range from 5-300 μm (11). The aim of this work is to prepare film of KT that was loaded in microsponges to be implanted at the posterior part of the eye (vitreous chamber) as alternative regimen to conventional dosage form (topical and intravitreal injection) to treat retinal complicated conditions to enhance patient compliance by reducing frequent dosing as well as side effects.

### MATERIALS AND METHODS

**Materials**

Ketorolac tromethamine (KT) was purchase from Provizer – India. Dichloromethane (DCM) was purchase from Fluka – Germany. Poly vinyl alcohol (PVA) and glycerol were purchase from GCC, U.K.

**Methods**

Implantable film base preparation

The implantable film base was prepared by dissolving (3 gm) of (PVA) in (20 ml) hot sterile deionized water, after that (1 gm) glycerol was added with continuous stirring.
Implantable film preparation
Solvent casting method was used to prepare implantable film in which (0.5 ml) of the prepared base solution was added to (0.5 ml) sterile deionized water containing certain amount of our previously prepared KT microsponges (containing 30% PLGA as a polymer and 0.05% PVA as a stabilizer which was prepared by double emulsion method w/o/w)\(^{18}\) equivalent to (6 mg) of KT then (0.5 ml) aqueous solution containing (3 mg) uncoated pure KT was added with continuous stirring (using magnetic stirrer) for 30 minutes at 1000 rpm then casting the mixture in circular mold with dimensions (0.6 cm × 0.3 cm). The casted film was left to dry for 24 hours at 40 ºC. After that, the same film was transferred to a jar (with high humidity) containing saturated solution of aluminum chloride for the next 72 hours then its weight was determined (Wt. i). The percentage moisture absorption (PMA) can be measured by the following equation\(^{19, 20}\):

\[
PMA = \frac{W_{t. f} - W_{t. i}}{W_{t. i}} \times 100\% \]

Weight variation
The weight variation was calculated by weighing three prepared films individually using digital balance and the average weight was calculated\(^{15}\).

Thickness
The thickness was measured by choosing five different points at the center and edges of the prepared film using Vernier caliper\(^{16}\). This test repeated for three prepared films.

Surface pH
The surface pH of the film was recorded by wetting it with (0.25 ml) deionized water then the electrode of pH meter was placed in contact with the film surface then the pH was determined\(^{17}\). Three prepared films were used for this test.

Folding endurance
The flexibility of the prepared film was determined by measuring its folding endurance manually. It was performed by folding the film more than one time at the same place till it cracks\(^{18}\). This test was applied for three prepared films.

Percentage moisture loss (PML) film measurement
The physical stability of the film in high humidity condition was studied by the percentage moisture absorption test. This test was done by keeping the film in a desiccator for 72 hours then its weight was determined (Wt. i). After that, the same film was transferred to a jar (with high humidity) containing saturated solution of aluminum chloride for the next 72 hours then the final weight of the film was determined (Wt. f). The percentage moisture loss (PML) was measured by using the following equation\(^{15, 20}\):

\[
PML = \frac{W_{t. f} - W_{t. i}}{W_{t. i}} \times 100\% \]

In vivo evaluation of KT implantable film
Weight variation
The weight variation was calculated by weighing three prepared films individually using digital balance and the average weight was calculated\(^{15}\).

In vitro release study
This test was done by using the modified Franz diffusion cell where the prepared film was placed in the donor chamber (21) and (4 ml) phosphate buffer pH 7.4 was added in the donor and the acceptor chamber separated by dialysis membrane (molecular weight cut-off (MWCT) 3500). Each chamber had capacity of 4 ml (equivalent to vitreous chamber volume)\(^{22}\). The diffusion cell was placed in the shaker water bath at 37 ºC and 25 rpm. At pre-specified time intervals (4 ml) was withdrawn from the acceptor chamber and replaced with fresh buffer solution\(^{23}\). Each withdrawn sample was analyzed at λ max 322 nm using UV-spectrophotometer.

In vivo test
Animals used
Rabbits were used for this study. The rabbits were retained in the animal house at the Pharmacy College, Mustansiriyah University under natural conditions (light-dark cycles and temperature range between 23 – 25 ºC) with free access to the food and tap water. This study agreed with the considerations of the ethical committee.

Animals grouping
The total number of animals used were (9) rabbits divided into (9) groups as shown in Table 1.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Group name</th>
<th>Animals no.</th>
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<tbody>
<tr>
<td>1</td>
<td>Control group</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Rabbit with induced ocular inflammation after one day</td>
<td>1</td>
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<tr>
<td>3</td>
<td>Rabbit with induced ocular inflammation after one week</td>
<td>1</td>
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<tr>
<td>4</td>
<td>Rabbit with induced ocular inflammation after two weeks</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Rabbit with ocular KT implanted film after one day</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Rabbit with ocular KT implanted film after one week</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Rabbit with ocular KT implanted film after two weeks</td>
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<tr>
<td>8</td>
<td>Rabbit with ocular KT implanted film after one month</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Rabbit with ocular KT implanted film after another one month</td>
<td>1</td>
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</tbody>
</table>
N.o. = number

- Group 1: Untreated rabbit eyes used as a control and the left eye was enucleated and subjected to histological investigation for the normal eye.

- Group 2: The left rabbit eye was used to induce inflammation with hyaluronic acid sodium salt (HASS) intravitreally (10 µg/µl in sterile saline) as inflammation inducing agent and the rabbit was previously anesthetized using ketamine hydrochloride IM injection (15 mg/kg) with topical instillation of tetracaine hydrochloride as eye drop 0.5% (24). After shaving the neck of the rabbit (until jugular vein be clear), 20% pentobarbital sodium (Dolethal) was injected I.V. to cause death and the eye was enucleated and subjected for histo-pathological investigation.

- Group 3: The rabbit eye was injected intravitreally with (10 µg/µl) HASS (as in group 2), the rabbit was sacrificed after one week and the eye was enucleated for histo-pathological investigation.

- Group 4: The rabbit eye was injected intravitreally with (10 µg/µl) HASS (as in group 2) the rabbit was sacrificed after two weeks and the eye was enucleated for histo-pathological investigation.

- Group 5: The rabbit eye was injected intravitreally with (10 µg/µl) HASS and at the same time by using microvitreoretinal blade a 5 mm circumferential scleral incision posteriorly to the corneal limbus was made (26). The prepared KT film was inserted intravitreally by partial folding to be entered through the incision using non-toothed forceps. Micrsurgical sponge was used to remove any vitreous gel prolapse then the incision was closed by simple interrupted suturing using 8-0 poly glycolic acid absorbable suture. The rabbit was monitored for any sign of visible irritation then it was sacrificed after one day and the eye was enucleated for histo-pathological investigation.

- Group 6: Same procedure was applied as in group 5 but the rabbit was sacrificed after one week of film implantation where the eye was enucleated and subjected for histo-pathological investigation.

- Group 7: Same procedure was applied as in group 5 but the rabbit was sacrificed after two weeks of film implantation where the eye was enucleated and subjected for histo-pathological investigation.

- Group 8: Same procedure was applied as in group 5 (where 10 µg/µl of HASS was injected intravitreally and at the same time the prepared KT film was inserted) but 10 µg/µl of HASS was injected intravitreally every 2 weeks and the rabbit was sacrificed after one month of film implantation where the eye was enucleated for histo-pathological investigation.

- Group 9: Same procedure was applied as in group 8 and the rabbit was sacrificed after two months of film implantation and the eye was enucleated for histo-pathological investigation.

Sampling

Each group of rabbits were scarified at the predetermined time periods to monitor drug effect on the rabbit ocular tissues. The whole eye was enucleated and immersed for 72 hours in a 10% formalin solution (for fixation) then transferred to different concentrations of ethanolic solutions to be dehydrated (once in 70%, 80%, 90% and twice in 100%, for two hours in each) then the eye was transferred to xylol (twice for 1 hour in each) and paraffin (twice for 2 hours in each). After that, the eye was transected vertically (craniocaudal transections) using microtome with 5-6 µm thickness then mounted onto slides and stained with iodine and haematoxylin. The stained slides were examined and pictured using Olympus BH-2 microscope and digital camera (27).

Statistical analysis

Independent sample t-test and one way analysis of variance (ANOVA) were used for statistical analysis. Statistically significant of the differences were considered when (p < 0.05). Spss 16 software was used for all data analysis.

RESULTS AND DISCUSSIONS

The prepared film of KT-microsponges (0.6 cm × 0.3 cm) containing appropriate amount of KT microsponges equivalent to (6mg) KT in addition to (3mg) uncoated pure KT was prepared to be implanted in the eye vitreous chamber, and their in-vitro evaluations showed the following:

Weight variation

The average weight of the prepared implantable films was (8.87mg ± 0.2) with variation less than (5%) which suggested that the implantable films had variation in weight within the acceptable limit (28, 29).

Thickness

The implantable film thickness was (0.3 cm ± 0.04) which indicated the homogeneity of films thickness that matched with the reported intraocular implantable devices thickness (30).

Surface pH

The surface pH of each implanted films was found to be in the normal ocular pH (7.34) which in turn indicated that the ocular implanted film will not cause any type of ocular irritation (31).

Folding endurance

The prepared implantable films were found to have folding endurance more than (300) times indicating excellent elasticity which is appropriate for packaging and storage (32). Percentage moisture absorption (PMA) and percentage moisture loss (PML) of the implanted film were measured since low moisture content affects positively on the mechanical strength and adhesive properties in addition to the friability of the film. Furthermore, drug release from the film will be affected by the moisture content since the moisture makes channels on the surface of the dosage form leading to drug release (33). The results showed that all implantable films had an average percentage moisture absorption (PMA) values
(3.9 ± 0.51) and average percentage moisture loss (PML) values (2.25 ± 0.3) and both values were within the acceptable range\(^{(38)}\).

**In vitro film release**

Figure 1 showed fast initial burst effect (40% cumulative drug release within the first day (due to the presence of pure KT) followed by continuous release up to (70%) within the first month and continued to give 90% within 3 months indicating the sustained release of the drug from its microsponges encountered in the film.

![Figure 1](image1)

**Figure 1:** The % cumulative release of KT microsponge implantable film with pure uncoated KT.

**In vivo test**

The enucleation (removal) operation of the eye after rabbit scarifying is shown in figure 2.

![Figure 2](image2)

**Figure 2:** The enucleation procedure of the eye rabbit.

After fixation and sectioning the eye (cutting the eye as craniocaudal sections), the sections were examined microscopically for tissues of ciliary body, cornea and retina in all the nine groups. Figure 3 shows the normal tissues of ciliary body, cornea and retina of the rabbit eye and as a control (group 1) for comparison to follow up the changes in these tissues in the other groups.

![Figure 3](image3)

**Figure 3:** Craniocaudal section of ciliary body, Cornea and retina tissues for normal rabbit eye (group 1).
After one day of hyaluronic acid sodium salt (HASS) intravitreal injection (group 2), there was mild hypersensitivity reaction in ciliary body with mild increase in infiltrated cells of the cornea while the histopathologic examination of the retina showed moderate vacuolation of ganglion cells layer as shown in figure 4.

After one week of inducing inflammation (group 3); the ciliary body showed epithelial hyperplasia which means there is severe hypersensitivity while there is marked increment in inflamed cell at the epithelial part of the cornea. Retina showed severe vacuolation of ganglion cells in ganglion cell layer (retinal ganglion cells) with infiltration of neutrophils (figure 5). These signs indicating acute inflammation.

![Figure 4](image1.png)

**Figure 4:** Craniocaudal section of ciliary body, Cornea and retina tissues of the rabbit eye after one day of inducing inflammation (group 2).

![Figure 5](image2.png)

**Figure 5:** Craniocaudal section of ciliary body, Cornea and retina tissues of the rabbit eye after one week of inducing inflammation (group 3).

After two weeks (group 4), the craniocaudal section of ciliary body showed the appearance of elastic fibers, vessels, and melanocytes (figure 6) while cornea consisted from avascular myofibrils stroma contained lymphocytes and plasma cells while retina showed vacuolation of ganglion cells layer with neutrophils and lymphocytes. All these indicated the sign of chronic inflammation.

From the above results, single intravitreal injection of HASS in the rabbit eye was enough to induce hypersensitivity within one day and acute inflammation within one week but within two weeks there was chronic inflammation, so the recurrent intravitreal injection of HASS every two weeks was adapted to maintain the chronicity of inflammation during the study.
After one day of KT film implantation (group 5) in the rabbit eye (that was previously injected with HASS) (figure 7) there was significant decrease in the inflammatory cells infiltration of retina and mild reduction in the hypresensitivy reaction in ciliary body and cornea tissues (in comparison with group 2, figure 4) and this was attributed to the quick anti-inflammatory action of pure KT powder encountered in the film which gave initial drug release. After one week of implantation of KT film in the rabbit inflamed eye (group 6), there was improvement in ciliary body tissues (in comparison with group 3) and the cornea showed decrease in the infiltration of inflammatory cells at the epithelial part while the retina showed mild vacuolation in ganglion cell layer with few numbers of neutrophils (figure 8) indicating the healing property of the implanted film for the acute inflammation.

After two weeks of implantation of KT film in the inflamed eye (group 7), there was marked decrease of inflammatory cells (figure 9) (lymphocytes, melanocytes and neutrophils) from ciliary bodies and cornea in addition to the retina tissue (in comparison to group 4; figure 6). These results indicated the effect of implanted film containing KT against...
chronic inflammation which could be related to the release of the drug from the encountered microsponges.

**Figure 9:** Craniocaudal section of ciliary body, Cornea and retina tissues of the inflamed rabbit eye after two weeks of KT film implantation (group 7).

The results of group 8 (after one month of KT film implantation with recurrent inflammation induction by HASS intravitreal injection every two weeks) showed no sign of chronic inflammatory cells in ciliary bodies, cornea and retina tissues (figure 10) as compared to the control group (group 1, figure 2) indicating the healing property of the implanted film of the chronic inflammation induced in the rabbit eye.

**Figure 10:** Craniocaudal section of ciliary body, Cornea and retina tissues of the inflamed rabbit eye after one month of KT film implantation (group 8).

After two months of KT implantation (group 9) (with recurrent inflammation induction by HASS intravitreal injection every two weeks), (figure 11) the of rabbit eye tissues (ciliary bodies, cornea and retina) were similar to the normal control group (group 1) which signalized the potency of KT in the implanted film as anti-inflammatory drug for acute and chronic inflammation where the presence of the drug powder in the prepared film was enough to give the relief of acute inflammation and the sustained release of the drug from the microsponges encountered in the implanted film was enough to be effective against chronic inflammation within the first two weeks and complete healing within the first month, while the eye returned normal (similar to the control) within two months indicating the ability of the implanted film to prevent recurrent inflammation.
The results suggested that the prepared KT film (implanted intravitreally) had a good anti-inflammatory activity that may be used to treat the postoperative cystoid macular edema that may happen after cataract surgery as well as to treat diabetic macular edema refractory to laser photocoagulation as alternative to repeated intravitreal KT injection in these complicated conditions, that may enhance patient compliance with reducing side effects.

CONCLUSION
The work succeeded to prepare film containing KT microsponges having good integrity and releasing the drug over prolonged time (3 months) that can be implanted in the vitreous chamber of the eye with no sigh of visible irritation on the outer surface of the eye and effective against acute and chronic inflammation with no recurrent irritation. The implanted film had the potentiality to improve patient compliance and provide an option of long term deliver of the drug to the vitreous chamber with reduced side effects.

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CONFLICT OF INTEREST
The authors report no conflicts of interest.

REFERENCES