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Preparation and Characterization of Timolol Maleate Ocular Films

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Abstract: The aim of the present work is to formulate suitable ocular films of timolol maleate for the treatment of primary open angle glaucoma which remains in the *cul-de-sac*, and achieve the sustained release of the drug during the desired period of the treatment. Method includes optimization of the formulation using various polymers and plasticizers.
The drug timolol maleate is used among other things in eye-drops for the treatment of glaucoma but topically applied ophthalmic solutions often exhibit low bioavailability due to rapid tear fluid turn-over and drainage.
In this article various physicochemical properties of timolol maleate is given ad formulation of film with its absorption maxima, standard curve and solubility profile is mentioned.
Key words: timolol maleate, glaucoma, ocular inserts, ocular films.

Introduction

Beta blockers reduce the production of aqueous humor. Examples include levobunolol, timolol, carteolol, betaxolol and metipranolol. Timolol came onto the scene in late 1978 and continues to be a major player in reducing IOP via reduction of aqueous humor production. It reduces IOP approximately 25% (as compared to 30% for the prostaglandins) in most patients; translating into a 1mm, 2mm or 3mm Hg reduction, depending on the baseline intraocular pressure.

Timolol was, and continues to be, widely used to manage glaucoma. Timolol is available in 0.5% and 0.25% concentrations. It is often inappropriately prescribed as 0.5% dosed b.i.d. While timolol has a sufficiently long half-life to allow it to be used once daily, such practice is rarely observed. Moreover, numerous studies and expert opinion consensus show that 0.25% is at the top of the dose response curve, meaning that increasing the concentration does not elicit greater therapy response. Possible side effects include difficulty breathing, slowed pulse, hair loss, lower blood pressure, impotence, fatigue, weakness, depression and memory loss.

Ophthalmic preparations such as solutions, thickened solutions, suspension and ophthalmic soft hydrogels (performed hydrogels and *in-situ* formed hydrogels) present some disadvantages. From these dosage forms the amount of drug delivered may vary due to the drop size of the instilled preparation or its volume which may not be uniform, consequently the dose of the active drug from these dosage forms will not be uniform and it will be incorrect. The viscous vehicles may cause blurred vision. The presence of additives such as preservatives and added polymers used as viscolysers, offer undesirable side effects. Alternatively solid ophthalmic dosage forms intended for ophthalmic use will be more effective due to less

frequent administration, with minimal amount of additives. The pulsed release of drug observed when conventional eye drops are used can be effectively avoided. In the present work, an attempt was made to prepare ocular films of timolol maleate using a solvent casting method[1-7].

In comparison with the traditional ophthalmic preparations (Eye drops) the solid ocular films present advantages such as[1, 8, 9];

- Administration of an accurate dose in the eye and thus a better therapy.
- Increasing in contact time and thus improving bio-availability.
- Possibility of providing a prolonged drug release and hence better efficacy.
- Reduction of systemic side effects and thus reduced adverse effects.
- Reduction of the number of administration and hence better patient compliance.

These inserts may be placed for front of the eye (FOTE) drug delivery in the lower cul-de-sac and less frequently in the upper vault or in the cornea. These are classified as insoluble and bio-degradable ocular inserts, depending upon the nature of the polymer used. The insoluble varieties are often referred as non-erodible ocular inserts[10-14]. The other class is soluble ocular inserts, which are completely soluble so that they need not be removed from the site of application. They can be broadly divided into two types, the first being based on natural polymer and the second on synthetic or semisynthetic polymer. Among the natural polymers collagen derivatives, gelatine, cross linked collagen, chitosan derivatives (chitosan base, acetylated chitosen) were cited as examples. Recently bio-erodible ocular inserts made from alginate, polyvinyl alcohol, hypromellose and gelatin was mentioned in patient literature. But the patent literature often conceals the details and does not provide the required information[15-19].

Among the bio-erodible polymers, cross linked gelatine, sodium alginate and pectin need further study to establish their use as ophthalmic solid drug devices. In the present work on attempt has been made to formulate bio-erodible ophthalmic inserts using gelatin as a film forming agent. Acid treated gelatine films will be prepared containing the active drug timolol maleate 0.5% w/w[20]. The casted films will be hardened by treating it with 10% w/v solution of Gluteraldehyde in isopropyl alcohol. The films will be prepared using 1.6mg, 1.8mg and 2.0 mg % w/v of gelatin on the basis of solvent (water) used and 4.0-7.0 ml %w/w glycerine on the dried weight basis of gelatin. And these films will be cut and hardened for a time period of 15, 30, 45 and 60 minutes in the hardening agents (i.e. Gluteraldehyde). The excess of Gluteraldehyde will be oxidized by dipping the films in a sodium metabisulphite solution[21-22].

The following experimental protocol was therefore designed to allow a systematic approach to the study.

1) Preparation of standard curve for timolol maleate in distilled water, phosphate buffer pH 7.4 and in artificial tear fluid.
2) Composition of timolol maleate ocular films.

Standardization Parameters for Timolol Maleate[23-26]

IUPSC name: 2-Propanol, 1- (1, 1-dimethylethyl) amino-3-[[4-(4-morpholinyl)-1, 2, 5-thiadiazol-3-yl] oxy]-, (S)-, (Z)-2-butenedioate (1:1) (salt).

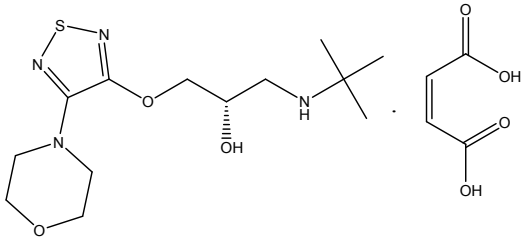

Fig.1. Structure of timolol maleate

Properties of TM

Molecular formula: $C_{13}H_{24}N_4O_3S.\ C_4H_4O_4$
Molecular weight: 432.49
pKa: 9.21

Physical properties

a.) **Appearance, color and odor:** Timolol maleate is a white, odorless, crystalline powder.
b.) **Melting point:**202±0.5°C
c.) **Solubility:** timolol maleate is soluble in water, methanol and ethanol; it is sparingly soluble in chloroform and propylene glycol. Timolol is practically insoluble in ether, cyclohexane and isohexane.

Mechanism of action: Blocks both β-1 and β-2 adrenergic receptors, reduces intraocular pressure by reducing aqueous humor production or possibly outflow; reduces blood pressure by blocking adrenergic receptors and decreasing sympathetic outflow, produces a negative chronotropic and inotropic activity through an unknown mechanism

Pharmacodynamics/Kinetics parameters

- Onset of action: $t_{1/2}$: 30 -90 sec.
- Hypotensive: Oral: 15-45 minutes
- Peak effect: 0.5-2.5 hours

- Intraocular pressure reduction: Ophthalmic: 30 minutes
- Peak effect: 1-2 hours
- Duration: ~4 hours; Ophthalmic: Intraocular: 24 hours
- Protein binding: ~10%
- Metabolism: Extensively hepatic (80%) via cytochrome P450 2D6 isoenzyme; extensive first-pass effect
- Half-life elimination: 2.5-5 hours; prolonged with renal impairment
- Excretion: Urine (15% to 20% as unchanged drug)
- Toxicity: LD_{50}= 1190 mg/kg (oral, mice), LD_{50}= 900 mg/kg (oral, rat)

Ultraviolet Absorption (λmax)

The solution containing 10 µg/ml of drug in artificial tear fluid (pH-7.4) was prepared and scanned over the wavelength range of 200nm to 400nm against artificial tear fluid as a blank using double beam UV spectrophotometer. The plot of absorbance vs. wavelength was recorded. UV spectrum of timolol maleate in artificial tear fluid pH-7.4 shows that the drug had λ_{max} of 294.0 nm that was exactly similar to the value reported.

Preparation of phosphate buffer pH 7.4 and artificial tear fluid

Preparation of phosphate buffer saline solution pH 7.4 (PBS pH 7.4) was prepared according to I.P.1996, Na_2HPO_3.1.38g, KH2PO4: 0.19g, NaCl: 8.00gand Distilled water q.s. to 1lit.

Composition of Simulated Tear Fluid[25]: NaCl: 0.670g, $NaHCO_3$: 0.200g, $CaCl_2.2H_2O$: 0.008g and Purified water q.s. to 100g.

Calibration Curve for timolol maleate

The calibration curves for timolol mleate were prepared in distilled water, phosphate buffer pH 7.4 and in artificial tear fluid.

Accurately weighed samples of 50mg of timolol maleate were dissolved in 100ml of phosphate buffer pH 7.4 and in artificial tear fluid respectively. 1ml of each of these solutions was diluted to 100ml with phosphate buffer pH 7.4 and artificial tear fluid respectively. The resulting stock solutions were of 5µg/ml. Aliquots of 1 to 10 ml of each of these solutions were diluted to 10 ml with their respective solvents to give 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50µg/ml concentrated solutions of timolol maleate.

The absorbance of prepared solutions of timolol maleate in phosphate buffer pH 7.4 and in artificial tear fluid were measured at 294 nm, in Thermospectronic UV 1 Spectrophotometer against the respective mediums as blank.

The absorbance data for standard curves are given in Table 1. The fig. 2 shows that the drug follows Beers' range in concentration range of 5-50µg/ml.

Table 1 : Calibration Curve of timolol maleate in Artificial Tear Fluid

Sr. No.	Concentration (µg/ml)	Absorbance
1	5	0.199 ± 0.005
2	10	0.227 ± 0.005
3	15	0.397 ± 0.008
4	20	0.419 ± 0.01
5	25	0.598 ± 0.007
6	30	0.646 ± 0.014
7	35	0.831 ± 0.011
8	40	0.862 ± 0.013
9	45	1.01 ± 0.024
10	50	1.065 ± 0.015

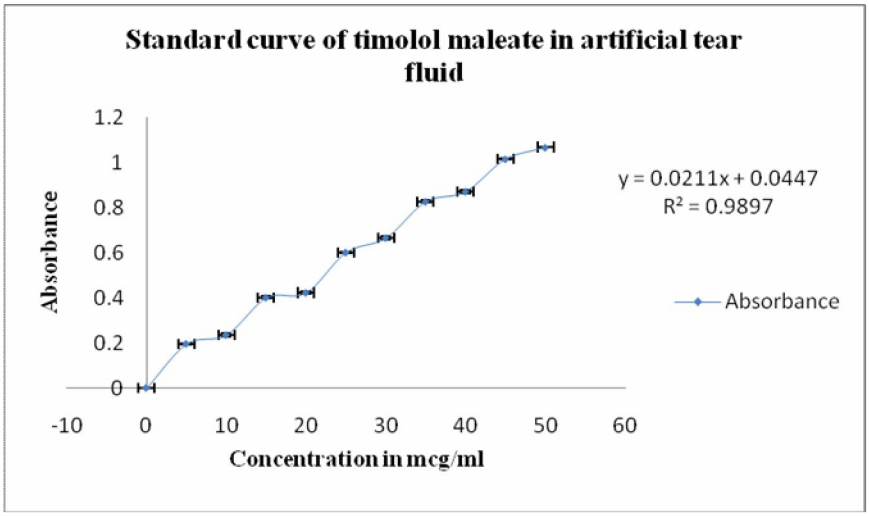

Fig.2. Standard curve of timolol maleate in artificial tear fluid

Method of Preparation of ocular films

The method of casting the films consists of the following steps:

1. Selection of a solvent system to dissolve the drug as well as the polymer.
2. Preparation of solution of polymer in the solvent system.
3. Addition of drug to the polymer solvent blend and mixing until a clear solution is obtained.
4. Addition of plasticizer, additives like preservatives and other additives if necessary.
5. Casting the film over a suitable substrate.
6. Gelling the film at a suitable temperature (10^{o}C).
7. Drying the gelled film.
8. Hardening of the film by cross linking agents.
9. Storage until use.

Process Details

In this system several variables and their effects on the film properties and the drug release was studied. In all the films the drug candidate was timolol maleate. In the systems timolol maleate concentration level was kept constant at 0.5% was per film. After preliminary experiments, which were carried out for finding the concentration of glatin, it was found that the concentration of gelatin required to form the film is more than 15% w/w.

A plasticizer is required to get flexible films of gelatin. In the present work glycerin were selected as plasticizer and was used in three different concentrations i.e., 40%, 50% and 70%. The films were prepared using Anumbra petridishes. As the polymer and the drug both are soluble in water, the same has been used as solvent (Table.2).

Formulation of Ocular Film of Timolol Maleate

Aim of the present work is to prepare the ocular films of timolol maleate using bio-degradable polymer (gelatine with glycerin).

Table 2: Composition of timolol maleate ocular films

Ingredients	Formulation codes								
	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8	TF9
Timolol maleate	0.5%	0.5%	0.5%	0. 5%	0.5%	0.5%	0.5%	0.5%	0.5%
Gelatin	16%	16%	16%	18%	18%	18%	20%	20%	20%
Glycerin	70%	50%	40%	70%	50%	40%	70%	50%	40%
Benzal Konium Chloride	0.002 %	0.002 %	0.002%	0.002%	0.002%	0.002%	0.002%	0.002%	0.002%
Distilled Water q.s.	100g m	100gm	100gm	100gm	100gm	100gm	100gm	100gm	100gm

In all these experimental batches the total weight of gelatin was kept i.e. 1.6, 1.8 and 2.0 grams in different combinations of formulations and the solution was placed in a Anumbra petridish (6cm internal diameter) gelatin and glycerin were weighed and dissolved in 75% quantity of the required distilled water and the mixture was heated at 60±5°C on a water bath until the entire polymer was dissolved. The drug and the remaining water along with the preservative (BKC) and plasticizer (glycerine) were added to make up the final weight. Out of these, 5.0gms of aliquots were poured on petridish using solvent casting method. The petridish were cooled at 10°C by placing on ice, until the films were gelled. The gelled films were taken out from ice and allowed to dry at room temperature for 72 hours. The petridishes were covered by an inverted funnel with cotton plug to prevent aerial contamination of the films during the drying time. The dried films were cut to the required size (7mm diameter) by cork borer and stored till use for *in-vitro* and *in-vivo* characterization viz. Uniformity of thickness, drug content and weight, water absorption characters, *in-vitro* and *in-vivo* release of drugs, accelerated stability study, sterility test and for further use.

Conclusion

The formulation of ocular films of timolol maleate is prepared with the objectives of increase contact time, prolonged drug release, decreased dose frequency and administration, improving therapeutic efficacy and thus may enhancing patient compliance.

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