

## Formulation and In Vitro-In Vivo Correlation of Timolol Maleate Ocular Insert

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### Abstract

The concept of in-vitro and in-vivo correlation studies was used in pharmaceutical research because a simple in-vitro release study on a drug product will be insufficient to predict its therapeutic efficiency. Therefore, correlation between in-vitro release behavior of a drug and its in-vivo absorption in rabbits must be demonstrated experimentally to reproduce therapeutic response. Aim of the study was to study the in vitro and in vivo evaluation and correlation of timolol maleate ocular insert. Timolol maleate ocular inserts were prepared by solvent casting method using guar gum in different proportions (0.25% w/v, 0.50% w/v, 0.75 % w/v, and 1.0% w/v). In vitro transcorneal permeation study was performed on goat cornea using modified Franz diffusion cell. The in vivo study was done using New Zealand albino rabbits and the in vitro in vivo correlation (IVIVC) was determined by plotting a graph of in vivo drug release was plotted against in vitro release to determine the correlation. The cumulative % drug releases from the formulation ranged from  $50.22 \pm 1.41$  to  $97.72 \pm 0.67$  over a period of 24 h. In vivo release of the timolol maleate from the optimized ocular inserts F2, through conjunctival cul-de-sac of rabbits was  $76.03 \pm 1.43$  at the end of 24 h. A high value of correlation coefficient ( $r^2=0.9965$ ) suggested good correlation between the in vitro-in vivo data of the timolol maleate ocular insert.

**Keywords:** Guar gum, IVIVC, in vitro transcorneal permeation study, ocular insert, timolol maleate

## Formulasi dan Korelasi In vitro-In vivo pada Timolol Maleat dengan Penyisipan Okular

### Abstrak

Konsep studi korelasi in-vitro dan in-vivo telah digunakan dalam penelitian farmasi karena studi invitro yang sederhana pada produk obat tidak cukup untuk memprediksi efisiensi terapi. Oleh karena itu, korelasi antara pelepasan in vitro obat dan penyerapan secara in vivo pada kelinci harus dapat didemonstrasikan secara eksperimental untuk menghasilkan respons terapi. Tujuan penelitian ini adalah mengevaluasi formulasi dan korelasi in vitro dan invivo pada timolol maleat dengan penyisipan okular. Penyisipan okular timolol maleat disiapkan dengan metode pemilihan pelarut menggunakan guar gum pada proporsi berbeda (0,25% w/v, 0,50% w/v, 0,75 % w/v, dan 1,0% w/v). Permeasi transkorneal in vitro dilakukan pada kornea kambing menggunakan modifikasi difusi sel Franz. Studi in vivo dilakukan pada kelinci albino New Zealand dan korelasi invitro invivo (IVIVC) ditentukan dengan pelepasan in vivo obat yang diplot pada grafik yang dihubungkan dengan pelepasan in vitro untuk menentukan korelasi. Pelepasan kumulatif obat (%) dari formulasi memiliki rentang antara  $50,22 \pm 1,41$  sampai  $97,72 \pm 0,67$  pada lebih dari periode 24 jam. Pelepasan in vivo timolol maleat dari penyisipan okular teroptimasi F2, melalui konjungsi kul-de-sak pada kelinci yaitu  $76,03 \pm 1,43$  pada akhir periode 24 jam. Nilai yang tinggi dari koefisien korelasi ( $r^2=0,9965$ ) menunjukkan korelasi yang baik antara invitro dan invivo data penyisipan okular pada timolol maleat.

**Kata kunci:** Guar gum, IVIVC, penyisipan okular, studi permeasi transkorneal in vitro, timolol maleat

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## Introduction

Delivery of medication to the human eye is an integral part of medical treatment. The delivery of drug to the site of action has been practiced since ancient times, which successively advanced in a variety of ophthalmic dosage forms.<sup>1</sup> In the design of a drug delivery system for the eye a balance must be struck between the limitations imposed by the physicochemical properties of the drugs, the limitations imposed by the anatomy and disease state of the eye, and the dosing requirements of the drug for that particular disease.<sup>2</sup>

The most prescribed conventional ocular dosage forms for the delivery of drugs are eye drops, eye ointments and suspensions have disadvantages like poor bioavailability due to rapid precorneal elimination, normal tears turnover and conjunctiva absorption, frequent instillation of concentrated medication, side effects due to systemic absorption of drugs, blurred vision due to presence of viscous vehicles. Furthermore, the drug level in the tearfilm is pulsed, with an initial period of overdosing, followed by a longer period of under dosing.<sup>3</sup>

In order to improve drawbacks associated with conventional dosage form, it is desired that an alternative way of administration is needed to enhance the bioavailability of drug. Consequently, numerous novel ophthalmic drug delivery systems were developed to achieve a higher bioavailability of drugs.<sup>4,5</sup> Among these formulations are in situ gelling polymers, microspheres, nanoparticles, liposomes and ocular inserts. Ocular inserts of polymeric materials which can release the drug at pre-programmed rate without interference with the normal vision can serve this purpose.

Timolol maleate is widely used as a topically applied  $\beta$ -adrenergic-blocking agent in ophthalmology to lower the intraocular pressure of glaucoma patients.<sup>6</sup> It is given

in divided dose several times in a day in the form of eye drop.<sup>7</sup> Eye drop preparations though widely used suffer from the drawback of rapid drainage of drug out of the eye or into nasolacrimal pathway due to rapid tear turnover resulting in loss or systemic absorption of the drug. While loss of drug results in compromised therapeutic efficacy, systemic absorption results in undesired side effect.<sup>8</sup>

Though extensive research work is reported on ocular inserts, it could be evidenced from literature that guar gum is not reported as a polymer for preparation of ocular insert. As guar gum is a polysaccharide and already used in eye drops as viscolizer, can be used as a polymer in ocular insert. Thus, the present research work was undertaken with a view to prolong the ocular residence time as well as contact between the eye and the drug by incorporation in an insert.

The IVIVC for a formulation is a mathematical relationship between an in vitro property of the formulation and its in vivo act. The in vitro drug release profiles commonly act as distinguished in vitro characteristic. Whereas, the in vivo act is elaborated by plasma drug profiles, these profiles are then treated mathematically to assess whether a correlation exists; a correlation can generally be expected when drug release from the formulation is the step controlling the subsequent absorption kinetics.<sup>9</sup>

## Methods

### Materials

Timolol maleate was obtained as a gift sample from Micro Labs Ltd., Bangalore, India. Guar gum was procured from Ases Chemicals, Jodhpur, India. Sodium chloride, sodium bicarbonate and calcium chloride dihydrate were purchased from S.D. Fine Chemicals, Mumbai, India. Dibutylphthalate was purchased from Loba Chemicals, Mumbai, India. All the chemicals used were

**Table 1 Composition of Polymeric Matrices for the Different Formulation**

	F1	F2	F3	F4
Timolol maleate (mg)	60	60	60	60
Guar gum (% w/v)	0.25%	0.50%	0.75%	1.0%
Dibutyl phthalate (w/w of polymer)	30%	30%	30%	30%
Purified water (mL)	100	100	100	100

of I.P. / A.R. or equivalent grade.

#### Preparation of ocular insert

The ocular inserts were prepared by the solvent casting method.<sup>10</sup> Four batches (F1–F4) of timolol maleate ocular inserts were prepared using different concentrations of guar gum (Table 1). Required amounts of guar gum were weighed and dissolved in distilled water by pouring guar gum gradually with vigorous stirring. The mixture was stirred for 2–4 h for complete hydration of guar gum. Required amount of plasticizer (dibutylphthalate – 30% w/w of the polymer) was added followed by the drug and stirring was continued to form a homogenous solution.

After complete mixing, the solution was kept overnight to remove any entrapped air bubbles. The solution (10 mL) was poured into glass moulds (35 cm<sup>2</sup>), which were then placed on a flat surface and were covered by an inverted funnel with cotton plug to prevent aerial contamination and to allow slow and uniform evaporation at room temperature for 48 h. The dried films so obtained were peeled from the casting surface and cut into an appropriate size (8 mm diameter) using a sterile stainless steel borer. An area of 0.50 cm<sup>2</sup> containing 85.6 µg of timolol maleate was used in all studies.

These formulations were sterilized separately by exposing to UV radiation for 90 min in a cabinet under aseptic conditions and were finally packaged in pre sterilized aluminium foil. The ocular inserts were placed in a desiccator until use.<sup>11</sup>

#### In vitro transcorneal permeation study

##### Corneal preparation

The whole eye ball of the sheep was obtained from a local butcher's shop within half an hour of slaughtering of the animal, and was transported to the laboratory in cold (4°C) normal saline (0.9% w/v NaCl) immediately. The cornea was carefully excised along with 2–4 mm of the surrounding scleral tissues and was washed with cold normal saline till it was free from proteins.

##### Permeation study

Fresh cornea was mounted by sandwiching the surrounding scleral tissue between the clamped donor and the receptor cells of modified version of a Franz diffusion cell in such a way that its epithelial surface (apical) faced the donor compartment and the endothelial surface faced the receptor compartment. The cell was placed on a magnetic stirrer in a holding position. The receptor compartment was filled with 11 mL of freshly prepared simulated tear fluid (pH 7.2) and stirred using Teflon-coated magnetic stir bar. The ocular insert was placed to the epithelial side of the cornea in the donor cell and stirring of the receptor fluid (jacketed with water at 32±1°C) was started. At appropriate intervals, 1 ml samples were withdrawn from the receptor compartment and the withdrawn sample volume was replaced with an equal volume of fresh simulated tear fluid to ensure sink conditions. The withdrawn samples were filtered and, diluted suitably with STF and analyzed spectrophotometrically by

measuring the absorbance at the  $\lambda_{\text{max}}$  of 294.5 nm. The study was carried out for about 24 h and was performed in triplicate.

#### Ocular irritation study

The in-vivo studies were performed in accordance with the Institutional Animal Ethics Committee (IAEC) of ACPTE, Mastuana Sahib, Sangrur (Protocol No.–ATRC/04/13 /CPCSEA/2013-98) constituted as per directions of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), under the Ministry of Animal Welfare Division, Government of India, and New Delhi.

Ocular irritation studies were performed according to the Draize Irritancy test as per OECD test guideline 405. The OECD guideline for eye irritancy test is currently the most valuable and reliable method for evaluating hazard or safety of a substance introduced into or around the eye. The ocular irritancy potential of a substance is evaluated by observing them for any redness, inflammation or increased tear production. Testing was carried out on adult albino rabbits weighting about 1.5 to 2.0 kg of either sex. All rabbits were maintained under 12 h light and dark cycles and were fed with green vegetables throughout the course of study. Food and water was allowed ad libitum. Six rabbits were used for testing the eye irritation potential of the ocular insert. The sterile

formulation was placed in the lower fornix of the rabbit eye while other eye served as a control. The rabbits were observed periodically for 24h for redness swelling and watering of the eyes.

#### In vivo Release Study

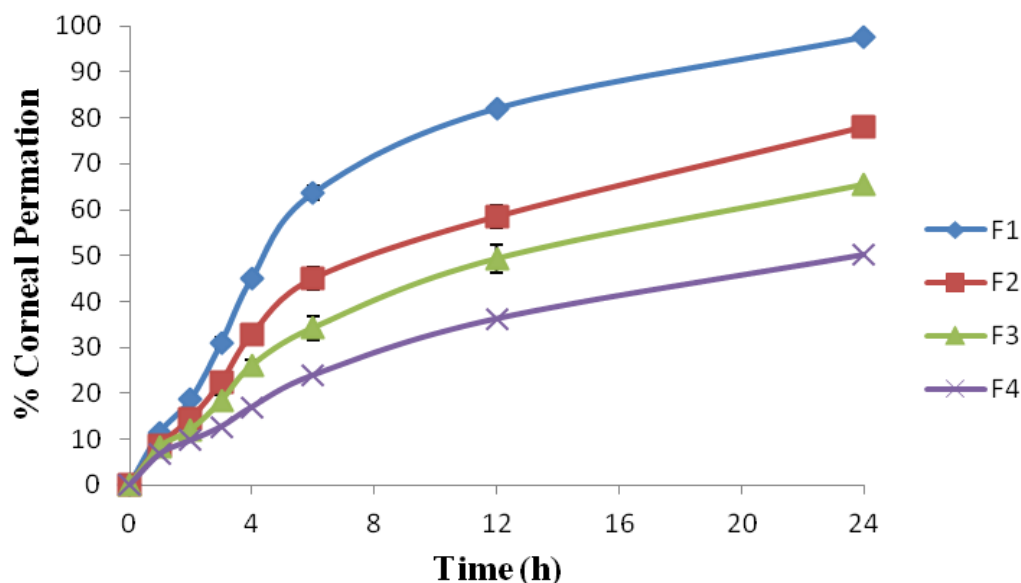
The results of ocular irritability and tolerability studies of all the formulations suggested that formulation F1 and F2 were well tolerated with no signs of any irritation. Formulation F3 and F4 showed redness in the sclera reason. Formulation F4 showed more redness as well as increase tear flow rate.

On the basis of release kinetics and ocular irritation study, F2 Formulation containing 0.50% guar gum was chosen for in vivo drug release study, as it prolongs the release of timolol maleate and shows no irritation to the eye. New Zealand albino rabbits weighing 1.5–2.0 kg, free of any signs of ocular inflammation or gross abnormalities were used for in- vivo studies. Each rabbit was kept in good hygienic condition in order to avoid vulnerability of any disease including ophthalmic type. The rabbits were housed singly in restraining boxes during the experiment and allowed feed with standard diet and water ad libitum. Free legs and eye movements are allowed.

Sterilized ocular inserts were placed in the lower cul-de-sac of each rabbit while the other eye served as a control. At periodic intervals (1, 2, 4, 6, 12, 24 h) the inserts were taken

**Table 2 In Vitro Transcorneal Permeation of Prepared Timolol Maleate Ocular Inserts**

Time (h)	In vitro transcorneal permeation (%)			
	F1	F2	F3	F4
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	11.49±0.63	8.73±0.36	8.30±0.64	6.81±0.37
2	18.67±0.89	14.51±0.64	12.06±1.11	9.73±0.42
3	31.00±0.30	22.51±0.85	18.34±1.64	12.73±0.53
4	45.11±1.29	32.84±1.23	26.05±1.35	17.03±1.19
6	63.65±0.66	44.95±1.480	34.21±1.31	23.98±1.73
12	82.14±0.56	58.48±2.38	49.27±2.53	36.28±1.66
24	97.72±0.67	78.04±2.54	65.39±3.05	50.22±1.41



**Figure 1 In Vitro Transcorneal Permeation of Prepared Timolol Maleate Ocular Inserts**

out carefully and analyzed for the amount of timolol maleate remaining in the insert. The amount of timolol maleate remaining was subtracted from the initial drug content of the drug, which gave the amount of drug released in the rabbit eye.

Observation for any fall out of the inserts was also recorded throughout the experiment. After one week of wash period the experiment was repeated for two times as before.

#### In vitro – in vivo correlation

The in vivo drug release study from timolol maleate ocular inserts F2 formulation was found to be in accordance with that of the in vitro drug release study. Hence, we tried

to correlate in vivo results with the in vitro percentage drug release. Here, a plot of in vivo drug release was plotted against in vitro release to determine the correlation as shown in Figure IV.

#### Results

The cumulative percent of timolol maleate released in vitro from the ocular inserts as a function of time is shown in Figure 1. The overall cumulative percentage drug release for formulation F1–F4 was found to be 97.72, 78.04, 65.39 and 50.22, respectively, at the end of 24 h, as shown in Table 2. The process of drug release from a guar gum

**Table 3 In Vivo Release Study of Timolol Maleate F2 Formulation**

Time	In Vivo Drug Release (%)	In Vitro Drug Release (%)
1	9.24±1.20	8.73±0.36
2	12.92±1.53	14.51±0.64
3	20.85±0.88	22.51±0.85
4	30.34±0.58	32.84±1.23
6	41.56±1.46	44.95±1.480
12	52.40±1.74	58.48±2.38
24	76.03±1.43	78.04±2.54



**Figure 2 Placing the Prepared Ocular Insert Under the Lower Fornix for In-Vivo Study of Formulation F2**

drug matrix involves water penetration into the dry matrix, hydration and swelling of the polymer, and diffusion of the dissolved drug in the matrix.

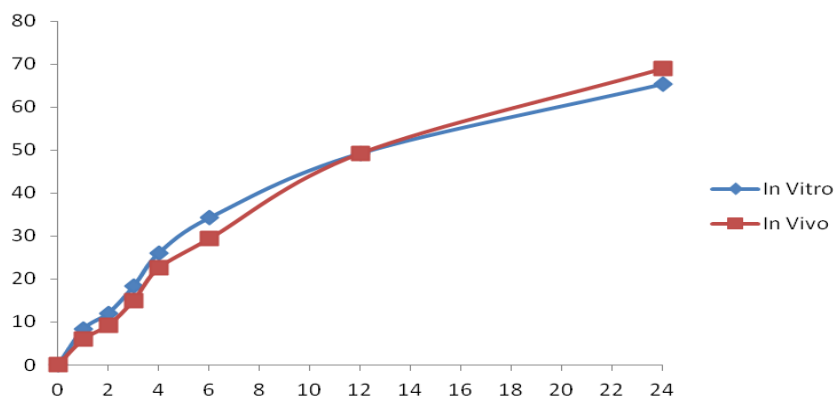
In vivo release of the timolol maleate from the optimized ocular inserts F2, through conjunctival cul-de-sac of rabbits was  $76.03 \pm 1.43$  at the end of 24 h as shown in Figure 3. The release profile demonstrated that ocular inserts could provide a sustained timolol maleate concentration in the cornea/tear film compartment for a prolonged period of time. Rabbits subjected for in vivo study did not show any signs of irritation, inflammation and abnormal discharge that confirmed the safety of the polymers used in the formulation. Also, there was complete absence of expulsion of films

from the rabbit eye during the entire study, which suggests that the dimension of the inserts (8 mm) was suitable for ocular use.

The in vitro and in vivo studies revealed that the formulation was capable of releasing the drug in concentration independent manner for the extended period of 24 hours. As it fulfilled many perquisites of Novel “Once a Day” delivery system and it may improve patient compliance.

### Discussion

The concept of in vitro and in vivo correlation studies was used in pharmaceutical research because a simple in vitro release study on a drug product will be insufficient to predict its therapeutic efficiency. So correlation between



**Figure 3 Comparison of In Vitro and In Vivo Release of F2 Formulation**



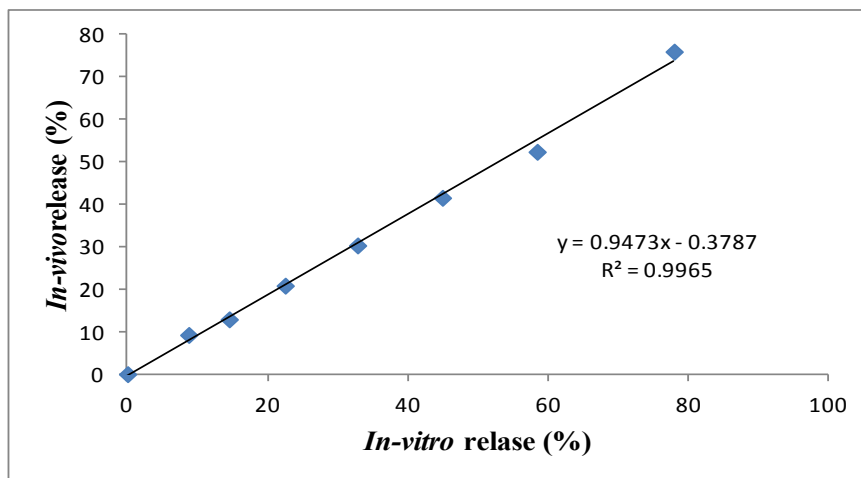


Figure 4 In vitro In Vivo Correlation Study of F2 Formulation

in vitro release behaviour of a drug and its in vivo absorption in rabbits must be demonstrated experimentally to reproduce therapeutic response. The knowledge of the relationship between the actual pattern of drug delivery and distribution or drug response is important when delivery systems with optimal input rates are to be developed for ocular therapy. From the scattered graph presented in figure IV stated that the correlation between in vitro and in vivo was strong and positive. The in vitro-in vivo correlation value was found to be 0.9965. There was a good correlation between in vitro and in vivo release data indicated correctness of the in vitro method followed and adoptability of the delivery system to the biological system, where it released the drug in concentration independent manner.

From biopharmaceutical standpoint, correlation could be referred to as the relationship between appropriate in vitro release characteristics and in vivo bioavailability parameters. United States Pharmacopoeia (USP) defined it as the establishment of a rational relationship between a biological property or a parameter derived from a biological property produced by a dosage form, and a physicochemical property

or characteristic of the same dosage form (USP, 2004). Food and Drug Administration (FDA) defined in vitro-in vivo correlation (IV-IVC) as a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant in vivo response. Generally, the in vitro property is the rate or extent of drug dissolution or release while the in vivo response is the plasma drug concentration or amount of drug absorbed (FDA, 1997).

## Conclusion

Correlations between in vitro and in vivo data (IVIVC) are often used during pharmaceutical development in order to reduce development time and optimize the formulation. A good correlation is a tool for predicting in vivo results based on in vitro data. IVIVC allows dosage form optimization with the fewest possible trials in man, fixes dissolution acceptance criteria, and can be used as a surrogate for further bioequivalence studies; it is also recommended by regulatory authorities.

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