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# Development of an Ion-Activated *In Situ* Gel System for Enhanced Ocular Delivery of Timolol Maleate

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article.

**Abstract**

**Background:** Glaucoma is a chronic eye disease that requires twice daily administration of eye drops. This study aims to develop a novel ion-activated in situ gel (ISG) formulation for once daily ocular administration in the management of glaucoma.

**Materials and Method**: Timolol maleate (TM) in situ gels, F1 to F12 were formulated using different concentrations and combinations of gellan gum (GG), sodium alginate (SA) and thermosensitive polymers of varying viscosities. The formulations were evaluated for physicochemical characteristics, gelling capacity, gelling temperature, drug content, rheological behavior, release kinetics, toxicity and irritability using the hen's egg test- chorioallantoic membrane (HETCAM) assay.

**Results:** All formulated ISGs were clear, colorless and free of particles. The pH of all formulations was between 5.27 and 6.81 which is within the acceptable range for comfort. The viscosity of the formulations at a shear rate of 100/s showed F1 (GG 0.2%, SA 0.5% w/v) had the lowest viscosity of 2 cp and F9 (GG 0.2% w/v, HPMC MV 0.6% w/v), had the highest viscosity of 202 cp at room temperature. Drug release studies showed sustained release of approximately 50% of the ISGs in 3 h compared to 30 mins for conventional TM solution.

**Conclusion**: ISGs formulated with the right combination of ion-sensitive polymers can potentially increase the residence time of TM in the eye and improve patient adherence to therapy.

**Keywords**: ion-sensitive, in situ gel, glaucoma, timolol maleate, sustained release.

## INTRODUCTION

Glaucoma is the main cause of preventable blindness and the second most common eye disease after cataract and is projected to affect 112 million people worldwide by the year 2040 (Rahić et al., 2021; Sekar & Chauhan, 2019). Glaucoma is a chronic eye disease characterized by elevated intraocular pressure above 25 mmHg and degeneration of retinal ganglion cells. Poor management or lack of treatment leads to visual impairment or blindness (Peng et al., 2012).

Nigeria accounts for 50% of glaucoma cases in Africa and 1 in 20 persons aged 40 years and above in Nigeria have glaucoma (Eboh & Eromosele, 2020). According to World Health Organization (WHO) 2022 Blindness and Vision Impairment report, vision impairment causes an annual global cost productivity loss of about US$411 billion, and persons aged 50 years and above are disproportionately affected. A global population of at least 2.2 billion people are visually impaired and 50% of these are due to preventable causes (World Health Organization, 2022). The ophthalmic market is

saturated with topical eye drops which make up about 90% of ophthalmic dosage forms. Frequent dosing required by this dosage form discourages patient adherence to dosage regimen and therefore leads to poor prognosis (Ubani-Ukoma et al., 2024).

Timolol maleate, the model drug for this study, is a well-known beta blocker used in the management of glaucoma with good safety profile and efficacy. A dosage regimen of twice daily instillation of timolol 0.5% has been reported to reduce intraocular pressure by 20 to 25% when administered appropriately. It is however contraindicated in patients with asthma, sinus bradycardia, cardiac failure and chronic obstructive pulmonary disease because of systemic drainage of the topical drug (Oddone et al., 2015). The nasolacrimal drainage of TM means that the drug is subjected to limited first pass metabolism by CYP2D6. In the event of poor metabolism by this enzyme, the above-mentioned side effects are exhibited. Due to its high systemic bioavailability of up 80%, the incidence of adverse drug reactions is high (Bollinger et al., 2023).

Eye drops are currently the most common dosage forms for the treatment of ocular diseases because of their ease of use (Lanier et al., 2020). They have a short resident time on the cornea and less than 5% of the administered dose is bioavailable (Maulvi et al., 2021). They are therefore instilled multiple times into the eye to increase their bioavailability (Ubani-Ukoma et al., 2022). Some of the medications are lost due to ocular barriers and small conjunctival sac volume while some drain into the systemic circulation through the lachrymal duct and cause adverse effects (Vaajanen & Vapaatalo, 2017). Consequently, there is the need for the development of patient-friendly and therapeutically effective dosage forms that would serve as appropriate alternatives to eye drops.

In situ gels (ISGs) are liquid dosage forms that exist as solutions on storage but quickly change to gels when they come in contact with the physiological environment of the body(Agha et al., 2023; Kurniawansyah et al., 2023). The sol-to-gel transformation occurs when the stimuli-responsive polymer content of the ISGs react to the pH, ion and/or the temperature of the tear fluid. With ISGs, the ease of administration of eye drops is retained, but the multiple instillations, poor adherence, systemic drainage and toxicity associated with frequent administration of eye drops are eliminated (Fathalla et al., 2022). The gels being more viscous than eye drops will be retained on the ocular surface for a longer period, creating enough time for absorption of the active pharmaceutical ingredients (API) leading to increased bioavailability and better therapeutic outcomes.

ISGs are formulated with ion-sensitive polymers, pH sensitive polymers and/or temperature sensitive polymers. In this study, we investigated the effect of two ion-sensitive polymers - sodium alginate (SA) and gellan gum (GG) and a viscosity-enhancing polymer, hydroxypropylmethylcellulose (HPMC) on the release kinetics of timolol maleate. SA is a polysaccharide polymer obtained from brown seaweeds - *Laminaria hyperborea, Ascophyllum nodosum,* and *Macrocystis pyrifera* or produced extracellularly from *Pseudomonas aeruginosa* and *Azotobacter vinelandii* (Chandra et al., 2022; Frent et al., 2022). They are composed of β-d-mannuronic acid (M) and α-l-guluronic acid (G) which influence its aqueous solubility and enhances their interaction with divalent cations ($Ca^{2+}$, $Mg^{2+}$) in the tear fluid to form ionotropic gels (Zhang et al., 2024). The gel-like structure formed increases the viscosity of the solution and the resident time of the formulation in the eye. Gellan gum is an exopolysaccharide derived from the bacterial cell of *Sphingomonas* bacteria and composed of α-L-rhamnose, β-D-glucose, and β-D-glucuronate units in the ratio of 1:2:1 (Abdl Aali & Al-Sahlany, 2024). This anionic polysaccharide transforms into a transparent gel-like structure when it comes in contact with hyaluronic acid and $Ca^{2+}$ in the tear fluid (Chandra et al., 2022).

Some studies have shown increased residence time of timolol maleate in situ gels formulations using GG alone (Yu et al., 2015), GG in combination with chitosan, a pH sensitive polymer (Shajari et al., 2024) and two pH sensitive polymers – carbopol and chitosan (Gupta & Vyas, 2010). Kral *et al*., 2024 also investigated the effectiveness of a combination of thermosensitive polymers – poloxamer 338 and poloxamer 188, and mucoadhesive agents – chitosan and carboxymethylcellulose in the formulation of timolol maleate ISG (Kral et al., 2024). In both studies, the authors confirmed the prolonged release and reduced systemic drainage of the formulations when compared with conventional TM eye drops. In this study, we investigated the controlled release effectiveness and tolerability of two biodegradable and generally regarded as safe ion-sensitive polymers – SA and GG in combination with HPMC of different viscosities, a viscosity enhancing polymer, to formulate a simple but novel ISG TM for the management of glaucoma. To the best of our knowledge, no study has been done on the combination of these two ion-sensitive polymers in the ocular delivery of TM.

## MATERIALS AND METHOD

### Materials

Timolol maleate, hydroxypropyl methylcellulose (HPMC) 4 – 6 cp, HPMC 40 – 60 cp, and HPMC 2600 – 5600 cp were purchased from Sigma Aldrich, USA. Gellan gum and citric acid 192.12 MW were obtained from Macklin Shanghai, China. Sodium alginate (low viscosity) and calcium chloride dihydrate were purchased from Loba Chemie PVT ltd, India. Sodium bicarbonate and di-sodium hydrogen phosphate were obtained from Molychem, Mumbai, India. Magnesium chloride, benzalkonium chloride and potassium chloride by Emsure, Massachusetts, USA. All other materials used were of analytical grade.

### Method

***Formulation of Timolol In situ Gel***

In situ gel formulations of F1 to F12 were made from different combinations gellan gum, sodium alginate and HPMC of varying viscosities – low viscosity 4 – 6 cp (HPMC LV), medium viscosity 40 – 60 cp (HPMC MV) and high viscosity 4000 – 6000 cp (HPMC HV). Benzalkonium chloride (BAK) was added at a concentration of 0.01% to serve as a preservative in all twelve formulations. This falls within the accepted concentration range of 0.002% to 0.02% (AlAani & AlNukkary, 2016). Each ISG was made according to the formula in Table 1 below:

TABLE 1: Composition of In Situ Gel Formulations

| ISG | Timolol Maleate *(% w/v)* | Gellan Gum *(% w/v)* | Sodium Alginate *(% w/v)* | HPMC LV *(%w/v)* | HPMC MV *(%w/v)* | HPMC HV *(%w/v)* | BAK *(%w/v)* |
|---|---|---|---|---|---|---|---|
| **F1** | 0.5 | 0.2 | 0.5 | 0.0 | 0.0 | 0.0 | 0.01 |
| **F2** | 0.5 | 0.2 | 1.0 | 0.0 | 0.0 | 0.0 | 0.01 |
| **F3** | 0.5 | 0.2 | 1.5 | 0.0 | 0.0 | 0.0 | 0.01 |
| **F4** | 0.5 | 0.2 | 0.0 | 0.5 | 0.0 | 0.0 | 0.01 |
| **F5** | 0.5 | 0.2 | 0.0 | 1.0 | 0.0 | 0.0 | 0.01 |
| **F6** | 0.5 | 0.2 | 0.0 | 1.5 | 0.0 | 0.0 | 0.01 |
| **F7** | 0.5 | 0.2 | 0.0 | 0.0 | 0.2 | 0.0 | 0.01 |
| **F8** | 0.5 | 0.2 | 0.0 | 0.0 | 0.4 | 0.0 | 0.01 |
| **F9** | 0.5 | 0.2 | 0.0 | 0.0 | 0.6 | 0.0 | 0.01 |
| **F10** | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.01 |
| **F11** | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 | 0.2 | 0.01 |
| **F12** | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 | 0.3 | 0.01 |
| **CTRL** | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.01 |

A 0.5% (*w/v*) timolol maleate solution was prepared aseptically by dissolving in distilled water with vigorous agitation and subsequent filtration. This served as a control sample for comparing with the formulated ISGs.

***Physicochemical characterization of ISGs***

The physical properties of the formulations such as color, clarity and transparency were assessed against a white and black background and the presence of unwanted particles in the solution was also noted. These were done to check for any physical or chemical reaction that may affect the integrity of the formulation.

***pH Determination***

The pH of the formulations was determined using an electronic pH device Mettler Toledo (Mettler-Toledo, Leicester, UK) by placing the pH electrode on the surface of 5 ml of the formulation after allowing for 1 minute equilibration on the formulation surface. The measurements were taken in triplicates and recorded as mean ± S.D.

***Determination of the rheological properties of the formulation***

The viscosities of the ISGs were determined using the DV2T Brookfield viscometer (AMETEK BROOKFIELD, Middleboro, MA, USA) at 25 ℃ using spindle size 3 at a torque of 20% and above. The spindle was rotated at a speed of 5, 20, 40, 60, 80 and 100 revolutions per minute.

***Drug content determination***

The drug content of each of the formulations was determined by measuring out 1 mL of the formulation and diluting it to 100 mL with distilled water. The timolol content of the formulation was determined using the UV/Visible spectrophotometry and an earlier determined calibration curve with an $R^2$ value of 0.9983. The wavelength of highest absorbance used was set at 295 nm.

***Gelling capacity determination***

The gelling capacity of the formulations was evaluated by introducing each formulation into 2 mL of simulated tear fluid in drops. The number of drops instilled before

gelation is observed is noted. The fewer number of drops required before gelation, the higher the gelation capacity. The time, temperature and pH of gelation was noted and the time for dissolution of the gel was also confirmed.

***In vitro drug release determination***

The drug release experiment was performed with 1 mL of each formulation using the Franz diffusion cell. The ISG formed was placed on the cellulose acetate membrane separating the donor compartment from the receptor compartment with STF solution maintained at 37℃ ± 0.5 and at pH 7.4 with constant stirring. The STF used was composed of NaCl 0.670 g, $NaHCO_3$ 0.200 g, $CaCl_2$ 0.008 g, $MgCl_2$ 0.005 g, KCl 0.138 g in 100 mL of distilled water. The cellulose acetate membrane was soaked in STF overnight before commencement of the release studies. Samples of 1 mL were withdrawn at pre-determined time intervals and replaced with fresh and equal volume of STF to maintain a sink condition. The amount of drug released was determined by UV/Visible analysis at a wavelength of 295 nm after appropriate dilutions.

***In vitro ocular irritability and toxicity test***

The irritability and toxicity of the optimized formulation was evaluated using fertilized Hen's egg test chorioallantoic membrane (HETCAM) assay. The HETCAM test was chosen as a suitable alternative to the Draize test in line with the 3 R principle of Replacement, Reduction, Refinement described on the Directive 2010/63/EU of the European Parliament and of the Council of September 22nd 2010 on the protection of animals used for scientific research (Díaz-Tomé et al., 2021) and the National Center of the Replacement, Refinement and Reduction of Animals in Research.

Freshly fertilized eggs obtained from the farm was incubated using an incubator for nine days. The eggs were rotated five times a day at 38 ℃ and 60% humidity. On Day 7, the eggs were candled to determine their viability.

After confirmation of viability, the eggshell around the air cell was gently removed and 0.3 ml of the optimized ISG was applied to the CAM surface. The eggs were observed for hemorrhage, lysis or coagulation over a period of 300 seconds or 5 mins. An irritation score (0 – 21) was calculated based on the equation below –

$$\text{Score} = \left(\frac{301\text{-sec } H}{300}\right) \times 5 + \left(\frac{301\text{-sec } L}{300}\right) \times 7 + \left(\frac{301\text{-sec } C}{300}\right) \times 9 \quad (1)$$

Where H – Hemorrhage, L – Lysis, C – Coagulation and sec is starting second. From the total score, each chemical is classified as non-irritant (0 – 0.9), slight irritant (1 – 4.9), moderate irritant (5 – 8.9) and strong irritant (9 – 21). Sodium chloride (0.9% NaCl) was used as the negative control while sodium hydroxide (0.1M NaOH) was used as the positive control.

***Stability Studies***

Three samples of optimized formulation F2 -1, F2 – 2 and F2 – 3 (GG 0.2% w/v, SA 1% w/v) were placed in a stability chamber of 40 ℃ and relative humidity of 75% for 3 months. One formulation was removed every month to check the physical appearance by visual analysis and drug content using the UV/Visible Spectrophotometer at a wavelength of 295 nm.

***Statistical Analysis***

All data were analyzed with Microsoft Excel 365 (Microsoft, Redmond, Washington, USA) and one-way ANOVA with Tukey Posthoc test (GraphPad Prism Statistical Software, San Diego, CA, USA). All analysis were carried out in triplicate and data were presented as mean ± standard deviation.

## RESULTS AND DISCUSSION

The results obtained confirmed the successful formulation of ISGs. An optimized in situ gel is meant to have a clear appearance with the right pH and viscosity to ensure that instillation of the formulation does not cause irritation. The in-situ gel should be sufficiently fluid to allow easy instillation in the eye before forming a gel on coming in contact with the eye physiological environment. This will ensure the right dosage is administered, and it remains long enough in contact with the eye for enhanced absorption into the eye (Destruel et al., 2017). Increased viscosity also ensures minimal drug loss through drainage (Pandey et al., 2021) and a non-irritant ISG will prevent reflex tearing that leads to loss of the active pharmaceutical ingredient.

### Physicochemical properties of ISGs

The formulated ion-sensitive ISGs were fluid at room temperature, clear in appearance and devoid of any particles (Figure 1).

Figure 1: Image of optimized In Situ Gel formulation (F2) of timolol maleate

Table 2 shows the physicochemical properties of the formulated ISGs such as gelation capacities, gelation temperatures and clarity.

Table 2: Physicochemical properties of formulated ISGs F1 – F12

| ISG | Gelation Temperature (°C) | Gelation Capacity* | No. of Drops | pH | Clarity |
|---|---|---|---|---|---|
| **F1** | 30.70 ± 1.56 | + | 4 | 5.27 ± 0.091 | Clear |
| **F2** | 30.35 ± 1.34 | +++ | 2 | 5.42 ± 0.067 | Clear |
| **F3** | 30.60 ± 1.41 | ++ | 2 | 5.35 ± 0.042 | Clear |
| **F4** | 33.10 ± 4.26 | + | 2 | 6.81 ± 0.156 | Clear |
| **F5** | 33.13 ± 5.82 | + | 2 | 6.74 ± 0.131 | Clear |
| **F6** | 33.83 ± 5.88 | ++ | 2 | 6.70 ± 0.162 | Clear |
| **F7** | 37.17 ± 3.51 | ++ | 2 | 6.50 ± 0.021 | Clear |
| **F8** | 38.23 ± 3.41 | ++ | 2 | 6.55 ± 0.035 | Clear |
| **F9** | 37.40 ± 5.45 | +++ | 2 | 6.42 ± 0.006 | Clear |
| **F10** | 38.40 ± 4.53 | + | 2 | 6.66 ± 0.010 | Clear |
| **F11** | 37.47 ± 2.23 | +++ | 2 | 6.44 ± 0.010 | Clear |
| **F12** | 38.23 ± 4.30 | +++ | 2 | 6.69 ± 0.006 | Clear |

*** Key**

| | |
|---|---|
| No gelation | - |
| Gelation occurred within seconds and remained for a few mins | + |
| Gelation immediate and remained for a few hours | ++ |
| Gelation immediate, and remained for an extended period | +++ |
| Thick gel formed | ++++ |

The pH of the formulations fell within the acceptable range for the eye which is between pH 4 and 8 (Baranowski et al., 2014). Within this pH range, the tear fluid can buffer the formulation and maintain the pH at 7.4 which is the normal pH of the eye. This will effectively prevent the instigation of reflex tearing and associated dilution of the formulation and subsequent loss via the nasolacrimal route or overflow of the conjunctival sac.

The process of sol-to-gel transformation was closely monitored by visualizing the procedure against a dark background for contrast (Figure 2). The time for gel formation and disappearance of the gel was recorded.

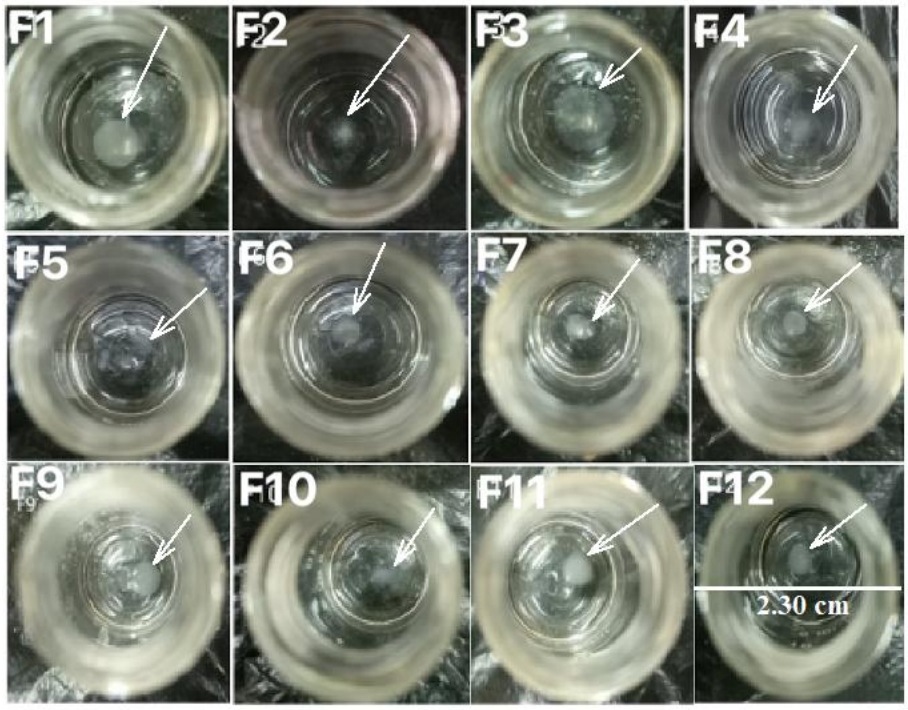



Figure 2: Images of i*n vitro* gelation of formulations F1 to F12 in 2 mL simulated tear fluid (STF)

The GG and SA imparted ion-sensitive responsiveness to the gels while the HPMC acted as a viscosity enhancing agent in the formulations. It can be observed from Table 2 that formulations F1 (GG 0.2%, SA 0.5% w/v), F4 (GG 0.2% w/v, 0.5% w/v HPMC LV), F5 (GG 0.2%, 1% HPMC LV) and F10 (GG 0.2% w/v, 0.1% w/v HPMC HV) had the lowest gelation capacity represented by the "+" sign. The low gelation can be explained by the low concentration and low viscosity of the SA and HPMC used in the formulation. Increasing the concentration of SA in the formulation and the use of the medium and high viscosity Hydroxymethyl propyl cellulose (HPMC MV and HPMC HV) resulted in an improved gelation capacity as exhibited by F2 (GG 0.2% w/v, SA 1% w/v), F9 (GG 0.2% w/v, HPMC MV 0.6% w/v), F11 (GG 0.2% w/v, 0.2% w/v HPMC HV) and F12 (GG 0.2% w/v, HPMC HV 0.3% w/v). Formulations F3 (GG 0.2% w/v, SA 1.5% w/v), F6 (GG 0.2% w/v, HPMC LV 1.5% w/v), F7 (GG 0.2% w/v, HPMC MV 0.2% w/v), and F8 (GG 0.2% w/v, HPMC MV 0.4% w/v) all exhibited acceptable gelation capacity but the gelation temperatures for 3 of the formulations varied based on the standard deviation in Table 2 except for F3. It was expected from the polymer contents of formulation F3 that it will exhibit a gelation capacity comparable to that of F2 but it did not. Though it exhibited immediate gelation as can be observed from the Figure 2, the gel formed did not last as long as that formed by F3 (Table 2).

**Rheological Properties of formulated ISGs**

The rheological properties of the ISGs are important in the selection of an optimized formulation for instillation into the eye. This is aimed at selecting a formulation that would ensure sustained release of the API without eliciting discomfort or irritation. The result of the viscosity measurement of the ISGs are shown in Figure 3 for all 12 formulations.

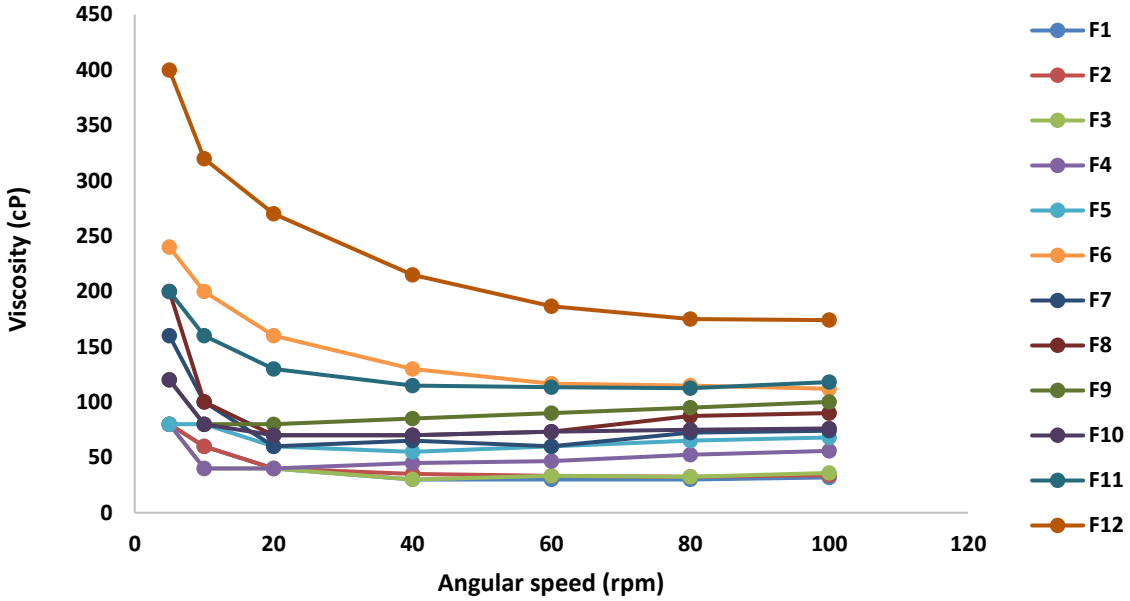


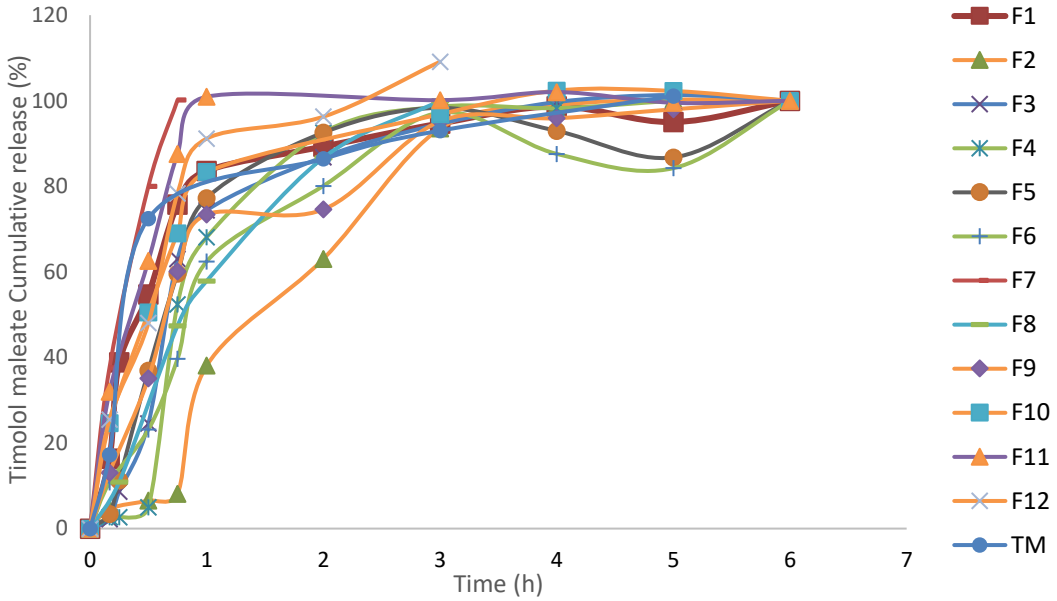

Figure 3: Formulations F1 – F12 rheograms of viscosity measurement against angular speed in revolutions per minute (rpm).

A topical pharmaceutical formulation is best developed as a shear thinning or pseudoplastic system. This implies a reduction in viscosity as the shear rate is increased resulting in a good spread. The rheograms in Figure 3 show that all formulated ISGs exhibited pseudoplastic flow as the viscosity of the formulations reduced with an increase in RPM. Pseudoplastic materials exhibit non-newtonian flow which is characterized by the disentanglement of the molecular structure and alignment of the constituent molecules in the direction of flow thereby reducing resistance to movement. Prior to administration, the ISGs remain in solution form but turn to gel on contact with the physiological environment of the tear fluid. This happens when the sodium alginate in the formulation interacts with the divalent calcium ions ($Ca^{2+}$) in the simulated tear fluid to form calcium alginate in situ (Makwana et al., 2016). This results in prolonged contact on the corneal surface and increased absorption of the API into the eye. Rotation of the eyeball after administration provides the shear stress which spreads the gel on the cornea and as shearing occurs, the gel thins out representing a reduction in viscosity as depicted in the rheogram.

**Drug release from ISGs**

The release kinetics of timolol maleate from the twelve ISGs was carried out over a period of six hours and compared to the timolol maleate solution which served as the control (Figure 4).

(a)




(b)

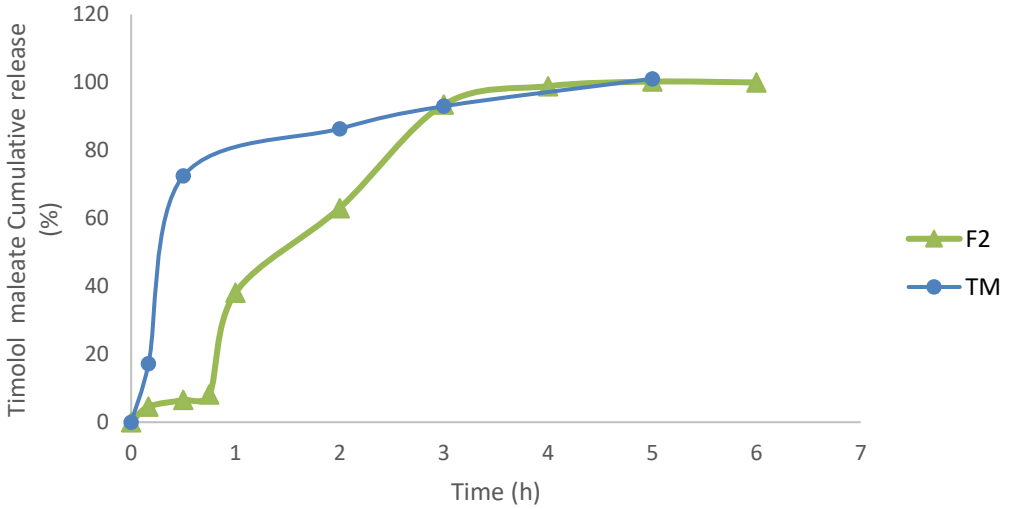



Figure 4: Percentage timolol maleate cumulative drug release from (a) Formulations F1 – F12 ISGs and TM solution (control) (b) Optimized ISG formulation, F2, and TM solution (control) release.

It was observed that formulations F7 and TM (control) had the fastest release time while F2 had the most

preferred release pattern of an initial lag time, followed by a gradual release over a period of three hours and tapered release.

Timolol maleate release from the various ISG formulations and conventional solution TM shows that the formulations duration varied depending on the concentration of the constituent polymers. Timolol maleate in all formulations was 60% released in an hour except formulation F2 (GG 0.2% w/v, SA 1% w/v) which was only 38% released in 1 h. The release curve of F2 showed an initial lag time where the drug was only 8% released within 45 mins. This was followed by a gradual release that spanned a period of 3 h after which there was a slow sustained release for another 3 h. This suggests that the drug will stay longer on the cornea, leading to higher bioavailability and a sustained release profile compared with the other formulations.

Formulations F1 (GG 0.2%, SA 0.5%) and F10 (GG 0.2%, 0.1% HPMCHV), F11 (GG 0.2%, HPMCHV 0.2%) and F12 (GG 0.2%, HPMCHV 0.3%) were over 80% released in 1 h. The fast release by F1 may be attributed to the low concentration of sodium alginate in the formulation. Formulations F10, F11 and F12 all made with high viscosity HPMC and are expected to exhibit prolonged release however, the release was very fast because of the low concentration of HPMC used. In contrast to the optimized ISG, the TM solution was 72% released in just 30 mins which confirms the requirement for frequent administration of eye drops while the F2 formulation may be administered once daily. The shear thinning property and sustained release from the ISGs observed in this study are similar to that made by Yu et al. and Shajari et al. who both used GG along with other polymers to investigate the sustained release of timolol maleate (Shajari et al., 2024; Yu et al., 2015).

The release data of the formulations F2, F9, F11 and F12 were fitted to different kinetic models because of their high gelling capacity (Table 2) to determine their release pattern based on the kinetic model that has the highest coefficient of correlation. Table 3 indicates that the ISGs have the highest correlation coefficient when fitted to the Korsemeyer-Peppas model which has a regression line value of 0.9584, 0.9924, 0.9982 and 0.9986 respectively and an n-value greater than 1 except for F2 with an n-value of 0.7406

.

Table 3: *In vitro* release kinetics data of ISGs with high gelling capacity

| ISG | Zero order | First order | Higuchi | Korsemeyer-Peppas | |
|---|---|---|---|---|---|
| | | | | $R^2$ | n |
| **F2** | 0.9398 | 0.9312 | 0.9505 | 0.9584 | 0.7406 |
| **F9** | 0.7103 | 0.4871 | 0.8792 | 0.9924 | 1.0565 |
| **F11** | 0.4932 | 0.9467 | 0.9194 | 0.9982 | 1.2340 |
| **F12** | 0.7225 | 0.9284 | 0.9018 | 0.9986 | 1.1466 |

This implies that formulations F9, F11 and F12 exhibited drug release by a combination of swelling and erosion while F2 release was by non-fickian diffusion.

***In vitro* toxicity and irritability test**

The toxicity and irritability potential of the optimized ISG, formulation F2 was assessed using the HETCAM test. The results obtained showed that the formulation was non-toxic and not irritable when exposed to the chorioallantoic membrane of the fertilized eggs (Figure 5).

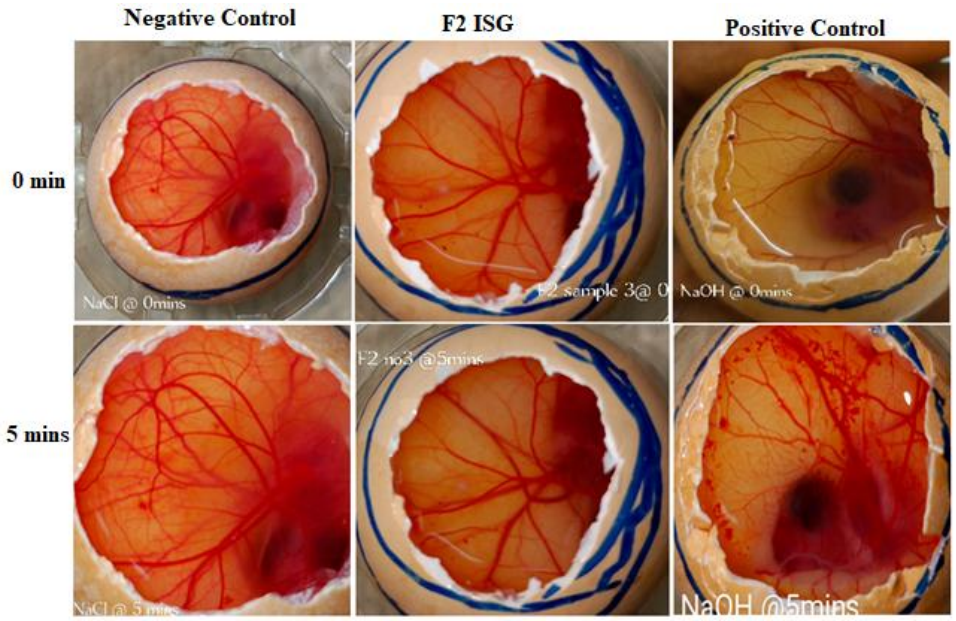



Figure 5: HETCAM test showing the CAM (chorioallantoic membrane) surface of fertilized eggs at 0 min and 5 mins after contact with 0.9% NaCl (negative control), ISG F2 (optimized ISG) and 0.1M NaOH (positive control)

The irritation scores for F2, the positive control and the negative control were determined using equation 1 and the time of observed reactions are shown in Table 4.

Table 1: HETCAM Irritation Score

| **Formulation** | **Observed Time (secs)** | | | **Irritation Score** |
|---|---|---|---|---|
| | **Lysis (L)** | **Hemorrhage (H)** | **Coagulation (C)** | |
| F2 | - | - | - | 0 |
| 0.1M NaOH | 5 | 7 | 7 | 20.62 |
| 0.9% NaCl | - | - | - | 0 |

Based on the observations, 0.1M NaOH (positive control) showed a high score of 20.62 confirming it is a strong irritant while 0.9% NaOH (negative control) and F2 with an irritation score of zero confirmed that they are non-irritant. In contrast to the images of the negative control 0.9% w/v NaCl and F2, the positive control showed hemorrhage, lysis, coagulation. An irritant formulation would cause redness, itching and tearing. This would increase the discomfort associated with drug administration and lead to increased drug loss by tearing.

**Stability studies**

The three F2 formulations did not exhibit a change in physical appearance by physical observation. However, the difference in drug content of the F2 – 3 formulation representing the optimized ISG left in the stability chamber for 3 months was significantly different from that of formulations F2 – 1 (1 month) and F2 – 2 (2 months). This was indicated by the UV absorbance values taken after removal of each of the samples from the stability chamber (Figure 6).

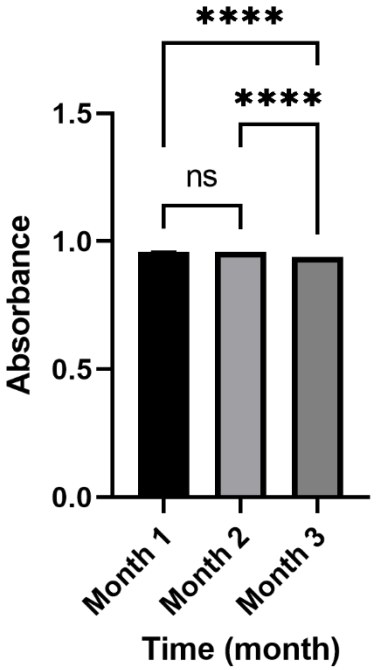



Figure 6: One-way ANOVA stability study results of F2 – 1, F2 – 2 and F2 – 3 in situ gel formulations. NB: **** depicts significant value of $p < 0.0001$ and ns – not significant.

The optimized formulation remained stable for 2 months under accelerated stability conditions of 40℃ ± 2℃ and 75%RH ± 5℃ and there was a significant change in concentration after the third month. The same formulation stored in the refrigerator at 4 ℃ remained stable throughout the period of the study. This implies that the shelf life of the formulation would be prolonged when stored in the refrigerator. Stability study carried out by Giri et al. showed the optimized in situ gel composed of gellan gum, sodium alginate and the API monocaprin remained stable for a month at different storage conditions – 4◦C ± 1℃, room temperature 22–25 ℃ ± 1 ℃, and temperature in the incubator 37 ± ℃ (Giri et al., 2023). In the study, the concentration of SA was kept constant at 0.16 while GG was varied from 0.083 to 0.66 and no preservatives were added to the eight formulations developed. Therefore, while both studies confirm the potential for stability of in situ gels with gellan gum and sodium alginate, the current study extends stability assessment to a longer duration (up to three months) and under more stringent accelerated conditions.

## CONCLUSION

Timolol maleate eye drops are effective dosage forms for the management of glaucoma, but it has a major challenge of unwanted systemic side effects, uneven dosing and requires frequent application. A novel, simple and safe in situ gel formulation of TM with SA and GG in the right combination has been developed and is a potential alternative to the TM eye drop. Further study in an *in vivo* model is needed to confirm the efficacy of the optimized formulation.

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