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In-Situ Gelling Ophthalmic Formulations for Sustained Release and Enhanced Ocular Delivery of Fluconazole

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***Abstract**: The present work describes formulation and evaluation of ophthalmic delivery systems of an effective anti-fungal named fluconazole (FLZ) based on the concept of temperature-triggered in-situ gelation using poloxamer 407 (P407) and ion-activated in-situ gelation employing sodium alginate (ALG) in purpose of easy eye instillation as drops that form gels in the eye providing sustained drug release, prolonged eye contact, and improved ocular delivery. Plain in-situ gelling ophthalmic formulations were prepared using different concentrations of the utilized polymers to optimize the gelling capacity. FLZ formulations with good gelling capacity were evaluated regarding viscosity, pH, drug content, in-vitro release, and stability for 6 months at different temperatures. The optimized formula that provided a sustained release and the highest shelf life was then assessed with respect to its ocular bioavailability in rabbits. All formulations possessed pH tolerable by the eye, acceptable viscosity values and drug contents complying with pharmacopeial limits. In-vitro release results indicated a sustained drug release without any burst effect and non-Fickian release mechanism. The optimized in-situ gel based on P407 (15% w/v) and HPMC (2% w/v) significantly improved FLZ ocular bioavailability in rabbits compared to its solution as a control. Therefore, this formulation may be represented as a potential drug delivery system of FLZ with a sustained release and an enhanced ocular delivery.*

***Key words:** Fluconazole, ion-activated in-situ gelation, temperature-triggered in-situ gelation, poloxamer 407, sodium alginate, stability, ocular bioavailability.*

1. Introduction

In-situ gel forming systems have been widely investigated as vehicles for sustained drug release. This interest has been sparked by the advantages shown by *in-situ* forming polymeric delivery systems such as ease and reduced frequency of application, improved patient compliance, and comfort. *In-situ* gel formation occurs due to one or combination of different stimuli like pH change as in case of carbopol 934 (CP934) **[1]**, temperature modulation using polymers such as poloxamer 407 (P407) **[2]** and solvent exchange employing polymers as sodium alginate (ALG) **[3]**. Fungal keratitis is a serious disease that can lead to vision loss if not diagnosed and treated effectively **[4]**. Previous ocular surface disease and trauma are the leading causes of fungal infection in the cornea **[5]**. Prolonged chemo- or immune-suppressive therapy **[6]** and contact lens use **[7]** have been also reported as predisposing factors of cornea infections. Unfortunately, current therapeutic options are limited **[8]**. Oral therapy requires high doses of antifungal agents to reach the therapeutic concentrations at the site of action, which may cause unwanted side effects. The problem of short residence time of formulations on the eye surface may be overcome by use of *in-situ* gels. These systems are applied as solutions or suspensions and then undergo gelation after instillation due to physico-chemical changes in the eye **[9]**. This allows a reproducible administration of the formulation into the eye as drops and an *in-situ* phase transition to a gel on the corneal surface. This may improve the retention time of the formulation and, consequently, of the drug. Furthermore, solution application to the eye is well tolerated by patients, which could contribute to compliance with the regime.

Fluconazole (FLZ) is a bis-triazole compound that exhibited a broad spectrum antifungal activity. It is effective against many fungal species including Candida **[10]**. It acts as a fungistatic agent. The high penetration into the aqueous humor and low toxicity of FLZ made it a good candidate as a topical ocular antifungal agent **[11]**. The azole antifungal drugs inhibit biosynthesis of ergosterol, the major sterol found in the fungal cell membrane, which is essential to the regulation of membrane fluidity and integrity and to the fungal growth and proliferation **[12]**. Topical FLZ solutions in experimental Candida keratitis have proved to be effective with a good penetration into the cornea and aqueous humor **[13, 14, 15]**.

Therefore, the objective of this study was to formulate, optimize and evaluate different *in-situ* gels of the promising antifungal FLZ. Two methods of *in-situ* gel preparation, namely ion-activated gelation (ALG and HPMC) and temperature-trigger gelation (P407 and HPMC), were employed. The studied formulations were

evaluated with respect to pH, viscosity, drug content and *in-vitro* release. The stability study was carried out for 6 months at 30, 40, and 50°C. The formulation that showed a prolonged drug release and the highest stability was assessed for its ocular bioavailability in comparison to the drug solution as a control.

2. Materials and methods

Fluconazole (FLZ), poloxamer 407 (P407) and hydroxypropyl methylcellulose (HPMC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium alginate (ALG) was obtained from AppliChem, GmbH (Germany). Carbopol 934 (CP934) was purchased from BDH Chemical Ltd, GB (Liverpool, England). All other chemicals were of fine analytical grade.

2.1. Preparation of FLZ *in-situ* gels

Different *in-situ* gelling preparations of FLZ were prepared under aseptic conditions to ensure the sterility of the final products. Based on the gelling capacity results, optimal concentration(s) of each polymer (P407, ALG, and CP934) with HPMC were determined. The required amount of each polymer was dispersed in 45 mL phosphate buffer saline (PBS) containing 0.01% benzalkonium chloride (BKC) as a preservative and stirred till complete dissolution. FLZ (0.3% w/v) was dissolved in 45 mL PBS and HPMC was added. The two resultant solutions were mixed using a magnetic stirrer. The volume was adjusted to 100 mL and transferred to a dry, clean, and sterile glass bottle. *In-situ* gel of P407 was prepared by following the same procedure except that the polymer was added to a previously cooled PBS to 4°C **[2]**. The plain formulations used for assessment of gelling capacity were prepared at the same method except that FLZ was not added.

2.2. Gelling capacity evaluation of the plain formulations

For determination of the suitable polymer concentrations to form *in-situ* gelling systems, all the plain preparations were evaluated for gelling capacity and transparency at physiological conditions. The gelling capacity was determined by placing a drop of the prepared formulation in a glass vial containing 4 mL of a freshly prepared simulated tear fluid (STF) which was equilibrated at 35 ± 0.5 °C. The visual assessment of the gel formation, transparency of the formed gel, the time required for gelation, and the time taken for the formed gel to dissolve was done **[16]**. **Table 1** shows the compositions and gelling capacity of the plain formulations.

Table 1: Composition and gelling capacity of plain formulations

Preparation No.	Concentration (% w/v)				Comment
	P407	HPMC	ALG	CP934	
1	15	-	0.1	-	No gelation
2	15	-	0.2	-	No gelation
3	15	-	0.3	-	No gelation
*4	15	2		-	Gelation after 2 min and remained for 12 h
*5	15	3	-	-	Gelation after 3 min and remained for 24 h
6	15	0.5	-	-	No gelation
7	15	1	-	-	No gelation
8	15	1.5	-	-	No gelation
9	-	2	0.3	-	Gelation after 2 sec and dissolved after 5 min
*10	-	2	0.4	-	Gelation after 2 min and remained for 12 h
*11	-	3	0.3	-	Gelation after 2 min and remained for 12 h
12	-	1	-	0.1	No gelation
13	-	2	-	0.2	Gelation after 5 sec and dissolved after 10 min
14	-	3	-	0.3	Gelation after 5 sec and dissolved after 4-5 h

*The selected preparations were that formed good gels within a short time and remained in the gel form for a reasonable time.

2.3. Evaluation of FLZ ophthalmic *in-situ* gels

2.3.1. Viscosity determination

In-situ gels of FLZ were subjected to viscosity determination using rotary viscometer (Haake Inc., Germany) which was calibrated before use. The measurements of temperature-triggered *in-situ* gelling systems were done at two different temperatures, 25 ± 0.5°C and 37 ± 0.5°C, to study their temperature-induced gelation. The viscosity of ion-activated *in-situ* gelling systems was estimated for both ophthalmic solutions and gels formed after adding three drops of STF **[17]**.

2.3.2. PH measurement

PH values of the ophthalmic FLZ preparations were measured using pH-meter (Beckman Instruments fullerton, CA 92634, Germany) **[18].**

2.3.3. Drug content

One gram of each formulation was accurately weighed and placed in a tightly closed volumetric flask containing PBS of pH 7.4, then the volume was completed to 100 mL with the buffer. The content of each flask was shaken in thermostatically-controlled water bath maintained at 37 ± 0.5 ^{o}C for 15 min., and a volume of 10 mL was centrifuged. The supernatant was filtered and analyzed spectrophotometrically (UV/VIS spectrophotometer V-530, Jasco, Japan) at 261 nm for drug content. The study was done in triplicate.

2.3.4. *In-vitro* drug release

The drug release from the investigated ophthalmic formulae in STF was carried out according to the method adopted by Levy and Benita, **[19]**. Cellophane membrane (molecular weight cut-off of 14,000 Da.) was soaked in STF solution and dried before use. This membrane was then stretched over the open end of a glass tube having a diameter of 3 cm and made water tight by a rubber band. Two grams of each formula were weighed and thoroughly distributed on the membrane. To each tube, a volume of STF equal to 1.5 mL was added. The tubes were then immersed upside-down in a beaker containing 50 mL STF maintained at 37 ± 0.5^{o}C using a thermostatically-controlled water bath. The tubes height was adjusted, so that the membrane was just below the surface of the release medium. The whole assembly was shaken at 25 strokes per min. At predetermined time intervals of 5, 10, 20, 30, 60, 120, 180, 240, 300, 360, 420, 480,540, 600, 660, and 720 min., aliquots of 3 mL each were withdrawn and replaced with the same volume of STF. The released amounts of the drug from each formula were determined spectrophotometrically (UV/VIS spectrophotometer V-530, Jasco, Japan) at 261 nm. Corresponding non-medicated formulae served as blank solutions. The experiments were done in triplicate and the mean values of percentage drug released were calculated.

2.3.5. Kinetic modeling of drug release

In order to determine the drug release mechanism, *in-vitro* release data were analyzed according to zero-order, first order **[20]**, and diffusion controlled release models **[21]**. Korsmeyer-peppas model was also used to elucidate the release mechanism of FLZ from the selected formulations **[22]**. The release exponent (n) is the slope of the plot obtained by linear regression of log percentage drug released per unit surface area versus log time.

2.3.6. Stability study

The tested formulations, each one of 10 gm, were placed in air tight amber glass jars and stored in thermostatically-controlled hot air ovens (Gering model SPA-GELMAN Instrument No. 16414, Germany) at different temperatures of 30, 40, and 50^{o}C for six months. They were evaluated initially and at specified time intervals (1, 2, 3, 4, 5 and 6 months) for various parameters. General appearance including color, odor, and turbidity was examined monthly. Monthly measurements of pH, viscosity, and drug content were done as previously mentioned. The method of accelerated testing of pharmaceutical products based on the principles of chemical kinetics has been demonstrated in the literature **[23]**. To determine the kinetic order which describes the pattern of drug degradation if any, some function of drug concentration in each formula monthly determined against time at the three temperatures was analyzed according to zero-order and first order kinetics. The model with the highest correlation coefficient (r^2) was considered to be the best fitting one. After that, the rate constant (K) values for the drug decomposition at various temperatures were calculated using the slope of the linear plot of the fitting kinetic model. The logarithms of the specific rates of decomposition (log k) were then plotted against the reciprocals of the absolute storage temperature and the slope of the resulting Arrhenius plots was used to estimate the activation energy (E_a) employing the relation (slope = -E_a/2.303R). The value of K_{25} was calculated using the relation Log (k_2/k_1) = E_a (T_2-T_1)/2.303RT_2T_1 considering that k_1= $K_{25,\ and}$ K_2 can be the rate constant calculated at any of the used temperature. The value of K_{25} was then used to obtain a measure of drug stability under ordinary storage conditions (shelf life, $t_{90\%)}$ and half-lives ($t_{50\%)}$ through the relations ($t_{90\%}$ = 0.105/K_{25}) and ($t_{50\%}$ = 0.693/k_{25}) **[23]**.

Arrheniusequation:

$$logK = \log A - Ea/2.303RT$$

Where; K= Specific reaction rate constant at temperature (t).
A = Frequency factor
E_a = Activation energy (Cal./mole).
R = Gas constant (1.987 Cal./deg.mole).
T = Absolute temperature (^{o}C + 273).

2.4. Ocular FLZ bioavailability in rabbits

2.4.1. Animal study

Ocular bioavailability of FLZ from the optimized formula selected based on *in-vitro* release and stability studies was examined in comparison with FLZ solution (0.3% w/v) in PBS pH 7.4 as a control. A total of 30 male New Zealand white rabbits weighing 2-2.5 kg were used and randomly assigned into two treatment groups each of fifteen rabbits. Rabbits were housed under standard conditions of temperature 25 ± 1ºC and 55 ± 5% relative humidity with regular 12 h light/12 h dark cycles. They were allowed free access to a standard laboratory food and water for 8 days to accommodate prior to the study. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee of Faculty of Pharmacy, Mansoura University, Egypt. Each animal received 100 mg of each formulation in the cul-de-sac of the right eye while the left eye served as a control by application of the corresponding plain formulation. The lower eyelid was gently moved to spread the medicated gel on corneal surface with care to avoid eye irritation via touching the corneal surface. All rabbits were gently restrained in up-right position for approximately 5 min after dosing to prevent the animals from shaking their heads or pawing at the eyes. FLZ concentration in different eye tissues and aqueous humor was determined at 1, 2, 4, 6 and 8 h post-dosing. Three rabbits from each treatment group were sacrificed, both eyes from each rabbit were separated. Samples of aqueous humor, conjunctiva, cornea, and iris/ciliary body were collected, separately. Each sample was weighed, homogenized with 0.2 gm of finely ground glass, shaken with 2 mL methanol overnight for drug extraction, and finally filtered through 0.2 µm filter.

2.4.2. HPLC assay

All samples were assayed employing high-performance liquid chromatography consisting of CMB-20 Alite system controller, LC-20 AD pump, DGU 20A degasser system (Shimadzu, Japan) using a promosil C_{18} column (250 mm×4.6 mm, 3µm, Agela Technologies, USA) with UV detector. Data analysis was accomplished using LC Solutions Version 1.3 software. An assay based on a mobile phase of methanol and deionized water utilizing C_{18} column and ultraviolet detection has been reported **[24].** The mobile phase consisted of methanol and deionized water (60:40, v/v) that was filtered (0.2 µm) and degassed before use. Separation was carried out isocratically at a flow rate of 1.0 mL/min. Injection volume was 20 µL. UV detector was set at a wavelength of 210 nm for better sensitivity and separation of drug peak from those of control eye homogenate. All assays were performed at ambient temperature.

2.4.3. Pharmacokinetic parameters

Pharmacokinetic parameters were calculated for each rabbit according to a previously reported method **[25]**. The maximum drug concentration in eye tissues and aqueous humor (C_{max}) and the time required to reach it (T_{max}) were estimated from the eye tissue concentration-time curves. Also, the elimination rate constant (K_e) was calculated from the terminal linear portion of the eye tissue concentration-time profile by linear regression analysis. The biological half-life ($T_{1/2}$) was calculated according to the relation $T_{1/2} = 0.693/K_e$. In addition, the area under eye tissue concentration-time curve from 0-8 h (AUC_{0-8h}) was calculated using the linear trapezoidal methods. AUC was extrapolated to infinity ($AUC_{0-\infty}$) by adding AUC_{0-8h} to C_{last}/K_e, where C_{last} is the last measurable concentration of the drug after 8 h. The relative FLZ bioavailability was determined as the ratio of $AUC_{0-\infty}$ of the tested formulation to that of the control.

2.5. Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons. Statistical calculations were done using GraphPad prism-5 software (GraphPad software Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Gelling capacity of the plain formulations

Gelation was observed in case of systems containing each of the employed polymers (P407, ALG, and CP934) combined with HPMC at concentrations ≥ 2% w/v, **Table 1**. All formed gels were transparent. The time required for gelation ranged from 2 sec to 3 min. Preparation based on CP934 combined with HPMC showed no gelation or relatively rapid dissolution of the formed gels. While, those containing HPMC (2% and 3% w/v) with each of P407 (15% w/v) and ALG (0.3 and 0.4% w/v) required 12-24 h to dissolve. Therefore, the later preparations were selected to provide ophthalmic *in-situ* gelling preparations of FLZ, **Table 2**.

Table 2: Composition of FLZ *in- situ* gelling systems

Ingredients	Formulation code			
	Temperature- triggered *in-situ* gelling systems		Ion-activated *in-situ* gelling systems	
	F1	F2	F3	F4
FLZ	0.3	0.3	0.3	0.3
P407	15	15	-	-
HPMC	2	3	2	3
ALG	-	-	0.4	0.3
BKC	0.01	0.01	0.01	0.01
Water to	100	100	100	100

3. 2. Evaluation of FLZ *in- situ* gelling systems

3.2.1. Viscosity determination

Viscosity values of the prepared ophthalmic *in-situ* gels were determined, **Table 3**. For ion- activated *in-situ* gelling formulations based on ALG and HPMC (F3 and F4), viscosity values after dilution with STF were much higher than those determined before addition of STF suggesting the phase transition. ALG contains moieties of α-L-guluronic acid (G) which interact with the calcium ions in tear fluid to form three dimensional ionotropic hydrogel matrices **[26]**. Hence, liquid formulations containing ALG undergo rapid transition into the gel phase on exposure to divalent cations in tear fluid **[27]**. Viscosity of temperature-triggered *in-situ* gelling formulations increased on rising the temperature from the non- physiological temperature (25°C) to the physiological one (37°C), and the conversion of solution to semisolid gel was observed confirming *in-situ* gelling characteristics. This reversible sol-gel transition behavior at high temperature may be due to aggregation between polyethylene oxide (PEO) and polypropylene oxide (PPO) moieties of P407 to form micelles. When the concentration and the temperature of P407 aqueous solutions are above the critical values, the molecules will arrange to form micelles with a dehydrated PPO core surrounded by hydrated swollen PEO chains causing an increase in the viscosity **[28]**.

3.2.2. PH measurements

The mean pH values of *in- situ* gelling formulations were in the range of 6.88 ± 0.15 to 7.34 ± 0.45 which can be tolerated by eye **[23]**.

3.2.3. Drug content

As shown in **Table 3**, the percentage drug content in all *in-situ* gelling formulations was in the range of 100.25 ± 1.34 % to 102.64 ± 1.15 % complying with the pharmacopeial limits of 90 %-110% of the labeled claim [29].

Table 3: Viscosity, pH and drug content of FLZ *in-situ* gelling systems.

Formula code	Viscosity (mpa.s), mean ± SD				PH mean ± SD	Drug content (%w/w)
	Temperature- triggered *in-situ* gelling systems		Ion-activated *in-situ* gelling systems			
	25 ± 0.5℃	37 ± 0.5℃	Without STF	With STF		
F1	548 ± 24	978 ± 25	_	_	6.88 ± 0.15	100.25 ± 1.34
F2	603 ± 66	1026 ± 67	_	_	6.92 ± 0.56	101.10 ± 1.68
F3	_	_	635 ± 17	1220 ± 102	7.23 ± 0.87	102.64 ± 1.15
F4	_	_	706 ± 38	1331 ± 145	7.34 ± 0.45	100.77 ± 1.48

3.2.4. *In-vitro* drug release

Figure 1 illustrates the release profile of FLZ from *in-situ* gelling systems compared to its control solution. In contrast to the control solution, the release profiles of FLZ from the tested ophthalmic *in-situ* gelling formulations indicated a sustained drug release free from any burst release that may cause toxicity. Such sustained release may be explained on the basis that the drug was diffused through the gel matrix or released from the eroded polymer network before delivery to the dissolution medium. The release of the drug from these formulations can be arranged as follows: F1> F2 > F3 > F4. The results of statistical analysis of drug *in-vitro* release revealed a significant difference between the control and each of F1, F2, F3 and F4 and between F1 and F4 ($p < 0.05$).

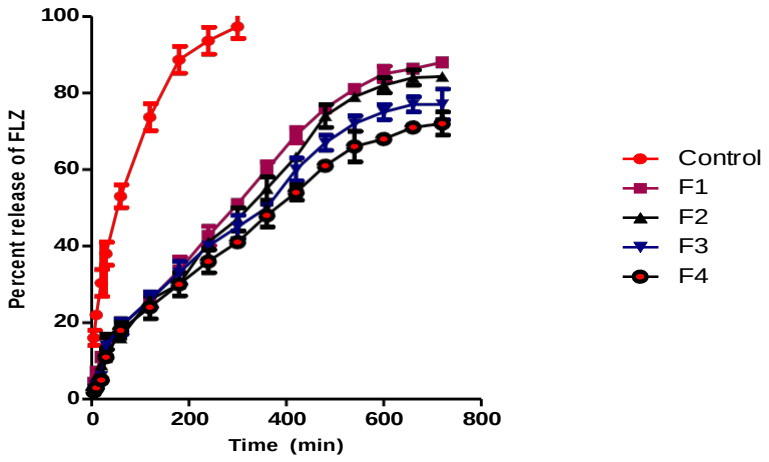

Figure 1: *In-vitro* FLZ release from studied *in-situ* gel formulations in STF pH 7.4 compared to its control solution.

3.2.5. Release kinetics

In-vitro drug release profiles could be best expressed by Higuchi's equation as the plots showed good linearity, **Table 4**. However, the analysis of the release data using Korsmeyer-Peppas equation indicated that the release exponents (n) for all formulations ranged from 0.599 to 0.736 suggesting non-Fickian or anomalous diffusion, **Table 4**. Therefore, the drug release from the formulation was not explained by a pure diffusion mechanism, but it was rather a mixture of both diffusion and erosion of the polymer matrix. Release from initially dry, hydrophilic glassy polymers that swell on water addition and become rubbery can be explained by anomalous diffusion.

Table 4: Kinetic analysis of FLZ release from *in-situ* gelling formulations.

Formula code	Correlation coefficient (r^2)			Release order	Korsmeyer-Peppas model		Main transport mechanism
	Zero order	First order	Higuchi model		r^2	n	
F1	0.974	0.818	0.983	Diffusion	0.992	0.599	Non-Fickian
F2	0.975	0.813	0.977	Diffusion	0.988	0.614	Non-Fickian
F3	0.963	0.723	0.989	Diffusion	0.981	0.685	Non-Fickian
F4	0.965	0.704	0.992	Diffusion	0.982	0.736	Non-Fickian

3.2.6. Stability study

All *in-situ* gelling formulations were found to be physically stable as they didn't show any color or odor changes during storage period at 30, 40, and 50°C. All formulations exhibited no drug precipitation during the whole period of stability study at the used temperatures. Monthly determined values of pH, viscosity, and drug content are represented in **Table 5**. The values of pH of the tested formulations were nearly unchanged during the whole storage period at the three temperatures. The viscosity increase of temperature triggered *in-situ* gels (F1 and F2) may be due to thermoreversibility of P407. This polymer has been reported to transform from a liquid state to a gel on temperature rise **[2].** Regarding FLZ content, at the end of the storage period (after six months), the drug content of all tested formulations (except F2 at 50°C) ranged from 90.01 ± 3.16% to 93.85 ± 2.04% complying with the pharmacopeial limits of 90-110% of the labeled drug **[29]**.

Kinetic analysis of the obtained data revealed that the degradation rates of FLZ in the tested formulations at different temperatures followed first-order model as indicated by r^2 values ≥ 0.918. The estimated values of half-life and shelf life are demonstrated in **Table 5**. Among the tested formulations, those prepared using P407 (15% w/v) and HPMC (2% w/v), F1, exhibited the highest shelf life of 14.6 month. Therefore, this formulation was selected for further investigation of FLZ bioavailability in rabbit's eyes.

Table 5: Viscosity, pH, drug content, half-lives and shelf lives of *in-situ* gel formulations after storage for six months at different temperatures

Storage temp.	Parameters	F1	F2	F3	F4
30 ^{O}C	Viscosity	998 ± 11	1042 ± 25	567 ± 75	588 ± 34
	PH	6.59 ± 0.89	6.70 ± 0.39	6.93 ± 0.79	7.08 ± 0.09
	Drug content	93.85 ± 2.04	92.31 ± 1.68	93.72 ± 3.88	93.35 ± 4.44
40 ^{O}C	Viscosity	999 ± 72	1052 ± 11	558 ± 46	567 ± 72
	PH	6.52 ± 0.46	6.54 ± 0.07	6.88 ± 0.08	7.00 ± 0.67
	Drug content	93.20 ± 2.40	90.78 ± 2.64	92.90 ± 3.67	91.58 ± 5.88
50 ^{O}C	Viscosity	1011 ± 66	1074 ± 34	544 ± 46	554 ± 10
	PH	6.44 ± 0.37	6.42 ± 0.83	6.68 ± 0.77	6.76 ± 0.58
	Drug content	91.86 ± 2.37	88.04 ± 1.08	90.28 ± 3.69	90.01 ± 3.16
Half-life (month)		96.52	70.86	76.32	79.66
Shelf-life (month)		14.62	10.73	11.56	12.06

3.3. Ocular bioavailability of FLZ in rabbits' eyes

The time-concentration curves describing FLZ bioavailability in aqueous humor and different eye tissues including conjunctiva, cornea and iris -ciliary body are represented in **Figure 2**. The pharmacokinetic parameters of FLZ in the tested formulation (F1) including C_{max} (µg/gm) , T_{max} (h), K_e (h^{-1}), $T_{1/2}$ (h), $AUC_{0\text{-}8\ h}$ (µg.h/gm), $AUC_{0\text{-}\infty\ h}$ (µg.h/gm) and the relative bioavailability were significantly ($p < 0.0001$) higher than those of the control solution, **Table 6**. From the obtained results, it is obvious that the tested formulation improved FLZ bioavailability in all eye tissues and aqueous humor compared to the control solution. FLZ bioavailability can be arranged in the order of cornea > conjunctiva > iris-cilliary body > aqueous humor, **Figures 2** and **3**. The superiority of the tested *in-situ* gel (F1) over FLZ solution in improvement of the bioavailability in rabbit's eyes was possibly due to the faster wash out of the drug solution by tears and the prolonged contact of *in-situ* gel with the eye surface in addition to the sustained drug release. The higher celecoxib concentrations at cornea followed by conjunctiva compared to iris and aqueous humor has been reported **[30].** The obtained results showed that T_{max} in case of FLZ solution was 2 h in all eye tissues and fluid after application versus 4 h for the tested formulation (F1), **Figure 2**. Similarly, higher $T_{1/2}$ values of the tested formulation (F1) compared to the control solution was noted.

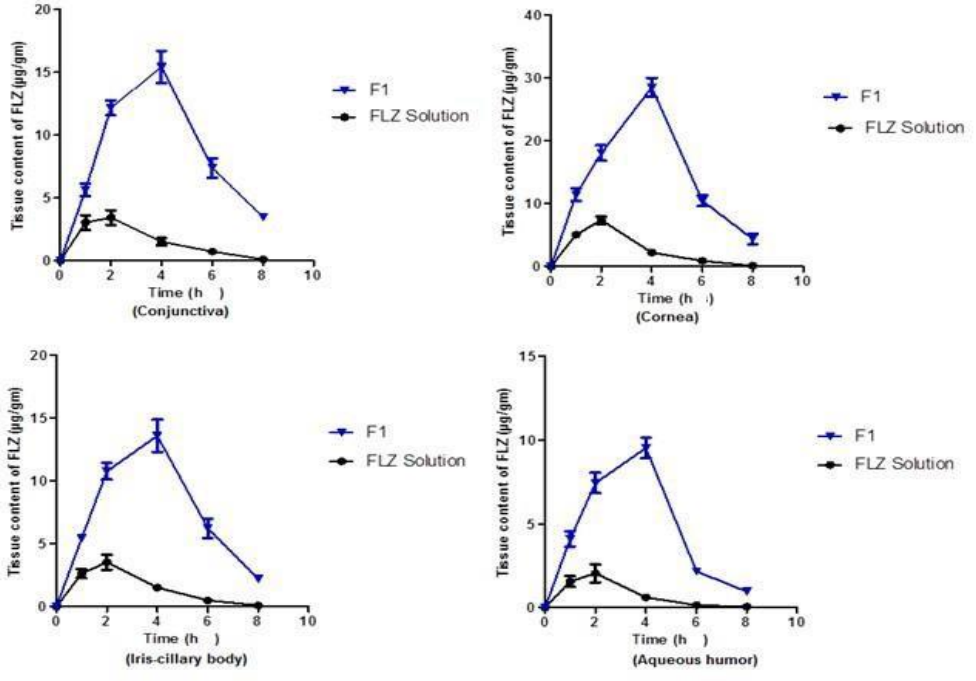

Figure 2: FLZ bioavailability in conjunctiva, cornea, iris-cillary body, and aqueous humor following topical application of *in-situ* gel (F1) and its control solution to rabbits' eyes.

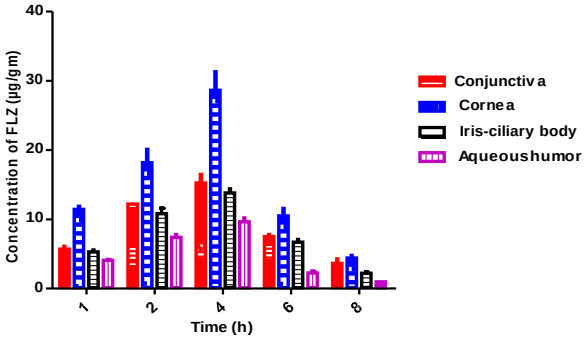

Figure 3: FLZ bioavailability from tested *in-situ* gel (F1) in eye tissues and aqueous humor.

Table 6: Pharmacokinetic parameters of FLZ after ocular application of *in-situ* gel (F1) compared to the control solution.

Tissue	Parameters	FLZ solution	F1
Conjunctiva	C_{max} (µg/gm)	3.44 ±0.27	15.12± 2.34*
	T_{max} (h)	2	4*
	K_e (h^{-1})	0.336 ±0.012	0.099± 0.007*
	$T_{1/2}$ (h)	2.10 ±0.08	7.08 ±0.32*
	$AUC_{0\text{-}8\,h}$	12.84 ±0.82	68.32 ±1.06*
	$AUC_{0\text{-}\infty\,h}$	13.58 ±1.31	71.32 ±2.43*
	Relative bioavailability	-	5.09 ±0.44
Cornea	C_{max} (µg/gm)	7.38± 0.96	28.84± 3.30*
	T_{max} (h)	2	4*
	K_e (h^{-1})	0.243 ±0.007	0.109 ±0.007*
	$T_{1/2}$ (h)	2.87 ±0.03	6.36 ±0.94*
	$AUC_{0\text{-}8\,h}$	22.57 ±0.42	117.13± 3.89*
	$AUC_{0\text{-}\infty\,h}$	24.26 ±1.72	144.27 ±4.22*
	Relative bioavailability	-	5.89 ±0.52
Iris-cillary body	C_{max} (µg/gm)	3.53 ± 0.34	13.76 ±2.22*
	T_{max} (h)	2	4*
	K_e (h^{-1})	0.357 ± 0.024	0.103 ± 0.005*
	$T_{1/2}$ (h)	1.98 ± 0.08	6.79 ± 0.26*
	$AUC_{0\text{-}8\,h}$	12.12 ± 0.33	63.57 ± 1.88*
	$AUC_{0\text{-}\infty\,h}$	12.94 ± 0.96	84.22 ± 3.64*
	Relative bioavailability	-	6.31 ± 0.33
Aqueous humor	C_{max} (µg/gm)	2.08± 0.37	9.62 ± 1.32*
	T_{max} (h)	2	4*
	K_e (h^{-1})	0.428 ± 0.032	0.096 ± 0.008*
	$T_{1/2}$ (h)	1.65 ± 0.07	7.27 ± 0.19*
	$AUC_{0\text{-}8\,h}$	6.30 ± 0.09	41.72 ± 0.92*
	$AUC_{0\text{-}\infty\,h}$	6.51 ± 0.08	51.06 ±1.76*
	Relative bioavailability	-	7.76 ± 0.28

* Significantly different at $p < 0.0001$ compared to the control solution

4. Conclusion

Ophthalmic *in-situ* gelling systems of FLZ were successfully formulated using each of P407 (15% w/v) and ALG (0.3% and 0.4% w/v) combined with HPMC (2% and 3% w/v), F1, F2, F3, and F4 respectively to provide an easy eye instillation as solutions with conversion into gels in the eye. These formulations showed pH tolerable by eye, acceptable viscosity values, pharmacopeial complying drug content and sustained drug release free from burst release. The optimized formula was that based on P407 (15% w/v) and HPMC (2% w/v), F1, as it showed the highest shelf life. A pronounced improvement of FLZ ocular bioavailability from the optimized formula (F1) compared to the drug solution may suggest it as a promising ophthalmic delivery system of this drug.

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