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Research Article

IN VITRO ENTRAPMENT AND RELEASE STUDIES OF LEVOFLOXACIN USING EPICHLOROHYDRIN-CROSSLINKED HYDROGEL

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ABSTRACT

Objective: This study aimed to optimize and evaluate the controlled release rate, ocular irritancy, and *in vitro* antimicrobial properties of levofloxacin entrapped in the epichlorohydrin-crosslinked hydrogel of sodium carboxymethyl cellulose (NaCMC) and gelatin.

Materials and Methods: Various parameters such as polymer ratio, amount of crosslinker, temperature, reaction time, swelling capacity, and percent drug loading were considered in Optimized levofloxacin hydrogel. Hydrogel preparations with higher amount of drug loaded were further analyzed to determine its *in vitro* drug release rate, ocular irritancy on New Zealand rabbits, and antimicrobial activities against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Optimized levofloxacin hydrogel (OLH) was then subjected to 3-month stability testing at 40 ± 2°C and 75 ± 5% relative humidity in which samples were withdrawn at the end of each month for analysis.

Results: Polymer groups with higher concentrations of NaCMC have higher swelling and drug loading capacities than those with higher gelatin concentrations. Meanwhile, qualitative analysis using differential scanning calorimetry, Fourier-transform infrared spectroscopy, and scanning electron microscopy verified the presence of levofloxacin in the epichlorohydrin-cross-linked hydrogel. Among the four polymer ratio, F3 was the optimized hydrogel with drug-loaded concentration of 99.50%, which was within the acceptable assay limit of 0.5% levofloxacin solution based on United States Pharmacopeia monograph. It followed the Higuchi kinetic model with a drug release mechanism of super case 2 transport indicating hydrogel swelling as a key factor for its controlled drug release. *In vitro,* antibacterial test against *P. aeruginosa* and *S. aureus* was sensitive to optimized levofloxacin hydrogel (OLH) with inhibitory diameter zones of 31.68 and 37.05 mm, respectively. Ocular irritancy test also showed that the OLH is non-irritating on installation in the cul-de-sac of New Zealand rabbits.

Conclusion: Optimized levofloxacin hydrogel was effective, non-irritating, and stable, which can be used as an alternative to conventional 0.5% levofloxacin ophthalmic solution.

Keywords: Cross-linked hydrogel, Levofloxacin, Ophthalmic preparation.

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INTRODUCTION

Misuse and overuse of antimicrobials impair their abilities to treat infections. It can also prolong illnesses which may lead to disabilities and deaths. Eye infections such as bacterial and fungal keratitis are one of the major causes of ophthalmic morbidities and visual loss with a reported incidence of 17–36% worldwide [1]. It is also a second predisposing factor of corneal opacifications which is commonly associated with soft contact lens users with a reported annual ulcerative keratitis incidence of 4–21/10,000 daily and extended wear users [2]. In this case, clinicians use varieties of antibacterial eye preparations and reports have shown that ocular pathogens show minimal bacterial resistance with fluoroquinolones [3] such as 0.3% ciprofloxacin and 0.5% levofloxacin which are efficient against aminoglycoside-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*.

Levofloxacin, a fourth-generation fluoroquinolone and an active L-isomer of Ofloxacin, is proven safe in treating bacterial conjunctivitis, keratitis, and other eye infections with known mechanism of inhibiting DNA gyrase and topoisomerase IV which are important steps for bacterial DNA metabolism [4]. It is registered as Quixin® (US), Oftaquix® (Europe), and Cravit® (Japan) with disadvantages similar to conventional eye preparations, such as rapid tear turnover and drug impermeability to corneal epithelium membranes similar to other conventional eye drops. Patient non-compliance is also an issue due to high frequencies of eye drop installations, which is a contributory factor to microbial resistance aside from improper dosage regimen, misuse, and overuse of topical antibacterial and extended duration of therapy.

In situ, hydrogel is a polymer liquid solution that undergoes a phase transition to semisolid gel due to changes in pH, temperature, and ion activation. On addition of a crosslinker specifically epichlorohydrin, its mechanical strength, and swelling properties will be enhanced, allowing diffusion of water-soluble drug into the hydrogel [5].

The main thrust of this study was to enhance the permeability and release rate of levofloxacin through drug entrapment in an epichlorohydrin-cross-linked hydrogel consisting of sodium carboxymethylcellulose (NaCMC) and gelatin.

MATERIALS AND METHODS

Materials

Epichlorohydrin and the reference standard levofloxacin were purchased from Sigma-Aldrich, while the high-performance liquid chromatography grade methanol for OLH analysis was purchased from Merck. Other analytical grade chemicals were acquired from Aishite Trading.

Methods

Compatibility testing

Thermograms of individual and combined ingredients were obtained using PerkinElmer differential scanning calorimetry (DSC) 400 in which

5 mg samples of Na CMC, gelatin and levofloxacin were pre-heated in sealed aluminum pans from 30 to 300°C at 10°C/min.

Hydrogel formulation

Various proportions of Na CMC and gelatin, as shown in Table 1, were dissolved with 50 mL distilled water. Dropwise of 10 µL of epichlorohydrin was added using a micropipette. The mixture was continuously stirred before it was spread evenly on glass plates. The semisolid mixture was heated inside the oven at 45°C for 240 min to facilitate the chemical reaction between the polymer and added crosslinker. Film thickness and weight of dried hydrogels were determined using a Vernier caliper and Kern Analytical Balance.

Drug loading

One milliliter of loaded hydrogel was then diluted with 100 mL simulated tear fluid (STF) pH 7.4. Five-milliliter aliquot samples were withdrawn, further diluted to 25 mL STF and then analyzed using ultraviolet-visible (UV-VIS) spectrophotometer (Unico SQ3802E) at 294 nm. Drug loading in the hydrogel was determined using the formula: Drug loading percentage = $t_1 - t_2$, where t_1 was the initial drug concentration in the solution and t_2 is the concentration in the supernatant solution.

Hydrogel characterization

A. Fourier-transform infrared (FTIR) spectroscopy – Functional groups present on dried OLH were analyzed using PerkinElmer Spectrum Two at 550-4000 cm^{-1} range at 4/cm resolution
B. Scanning electron microscopy (SEM):– Sufficient samples fixed with electroconducting glue were coated with gold powder, and its surface morphologies were analyzed using JSM-5310 (×1500, ×3500, ×7500, and ×10000 magnifications) with a maintained voltage of 10 kV
C. Swelling kinetics and maximum swelling degree – Pre-weighed and dried OLH samples were immersed in distilled water for 72 h at 25°C. Swollen hydrogels were dried with filter paper and weighed. Using the Dogadkin method, the percentage swelling of the hydrogel was calculated using the formula: $Q1=((m_s - m_o))/m_s)(100))$, where Q_1 was the swelling degree at time t, m_s was the weight of swollen hydrogel at time t, and m_d was the weight of the dry hydrogel. The maximum water absorbed by OLH was also calculated using the formula for equilibrium water content (EWC): EWC (%) = $((m_s - m_o)/m_s)(100)$, where m_s was the weight of swollen hydrogel at equilibrium [6].

In vitro drug release

A freshly prepared STF was prepared using the procedure given by Rajesh *et al*. (2012). The semi-permeable cellophane membrane was soaked for 24 h in STF. It was then wrapped in the stainless basket of the dissolution tester. One milliliter of OLH was transferred into the basket and suspended in a fiberglass vessel which acted as an acceptor chamber containing 500 mL STF. The temperature of the acceptor chamber was maintained at 37 ± 2°C with a constant stirring rate of 50 rpm. Four-milliliter sample was withdrawn every hour for up to 8 h and replaced with an equal volume of fresh diffusion medium [7]. Diluted aliquots in the diffusion medium were then analyzed at 294 nm wavelength using UV-Spectrophotometer. The same procedure was done using 1 mL marketed 0.5% levofloxacin solution (Oftaquix®). Data gathered were used to analyze the *in vitro* release rate of OLH using different kinetic models [8].

a. Zero-order kinetics – where percentage levofloxacin released in mg (Q) was plotted against time
b. First-order kinetics – where Q was plotted against time
c. Higuchi model – where Q was plotted against the square root of time
d. Hixson-Crowell model – where $Mo^{1}/^{3} - Mt^{1}/^{3}$ was plotted against time
e. Korsmeyer–Peppas model – where log Q was plotted against log time.

Antimicrobial activity

Using agar well method, an aliquot of 0.5 mL inoculums of test microorganisms (*S. aureus* ATC 25923 and *Pseudomonas aeruginosa* ATC 27853) were spread onto the agar plates and dried inside the biosafety cabinet. It was then punched aseptically with a sterile cork borer and filled with 100 µL of hydrogel (negative control), 100 µL of 0.5% levofloxacin solution (positive control), and 100 µL of OLH. The plates were incubated at 37°C and zones of inhibition were measured after 24 h using a Vernier caliper.

Ocular irritancy test

One to two drops of 0.5% proparacaine, HCl was administered on each eye 5 min before instilling 0.1 mL of OLH into the left cul-de-sac of three male New Zealand rabbit. The rabbits were monitored for 3 days for corneal opacity, reaction to light, eye redness, and swelling.

Accelerated stability studies

Three batches of the OLH were filled in glass vials securely closed to avoid moisture absorption. It was kept inside a stability oven with a maintained temperature of 40 ± 2°C and 75 ± 5% relative humidity for 3 months. Samples were withdrawn monthly to check the following: Organoleptic appearance, pH, viscosity, spreadability, gelling capacity, drug content, antimicrobial activity, and additional qualitative analysis using DSC, FTIR, and SEM to determine any possible changes that may have occurred within the 3-month storage period.

RESULTS AND DISCUSSION

Compatibility testing

Thermograms of each excipient were determined through the use of DSC (PerkinElmer DSC 400) in which initial peaks between 90° and 115°C was observed on most samples indicating desolvation. Meanwhile, levofloxacin was identified on DSC thermogram by its sharp endothermic peak at 238.68°C, gelatin at 262.55°C, and Na CMC at 90.76°C. When mixing levofloxacin with Na CMC, two sharp endothermic peaks at 228.65 and 236°C were observed indicating complex formation. However, a different endothermic peak at 214.80°C was observed when it is mixed with gelatin, and it even lowered on physical mixing of levofloxacin with gelatin and NaCMC with an evident broad peak at 211.50°C. According to Mutyaba *et al*. (2012), the mixing of drug and excipients may cause minor changes in the melting endotherm of a drug such as peak broadening or shifting toward the lower temperature. This is due to the lowering of the purity concentration of each component that does not necessarily indicate potential incompatibility.

A higher glass transition temperature at 220.34°C was observed with the crosslinked and loaded hydrogel at 0 month. This is due to the presence of covalent bond on addition of epichlorohydrin which leads to the formation of a mechanically stable hydrogel [9] in which its amorphous region requires a higher heat capacity [10] in the reversible transition of hard, brittle polymer into its molten rubber-like state [11].

Table 1: Formulation parameters in preparing optimized levofloxacin hydrogel (OLH)

Formulation code	Polymer concentration	Temperature (°C)	Reaction time (min)
F1	20% CMC+80% gelatin	45	240
F2	40% CMC+60% gelatin		
F3	60% CMC+40% gelatin		
F4	80% CMC+20% gelatin		

CMC: Carboxymethyl cellulose

Variables influencing the entrapment process

Different parameters, such as the polymer ratio, amount of crosslinker, temperature, reaction time, and swelling capacity, were considered in optimizing levofloxacin hydrogel. Based on the results shown in Table 2, hydrogels with greater amount of polysaccharides are heavier in weight, thinner, and have greater swelling capacity and thus, can be classified as super absorbent gel. This is due to a chemical reaction between the polymer mixture and anionic ion (F^-) present in epichlorohydrin which results to the formation of a permanent network which increases the mechanical strength and swelling capacity of the hydrogel. In addition, the carboxyl group present in NaCMC increased the electrostatic repulsions between the polymeric chains which permit fluid entry into the hydrogel, causing it to swell greatly [9].

Syneresis or the phenomenon of hydrogel shrinkage due to matrix coarsening or continuous squeezing-out effect, was observed on F1 due to decreased amount of NaCMC. This result is contrary to the findings of Buhus *et al*. (2009) in which hydrogel swelling degree in water is dependent on gelatin concentration [12].

Drug loading

The United States Pharmacopeia acceptance limit for the assay of 0.5% levofloxacin solution is 90–110% of its labeled claim in which the drug concentrations of the four groups were within the limit: F1 (99.03%), F2 (99.11%), F3 (99.50%), and F4 (99.51%). Cross-linked hydrogels with higher amount of Na CMC have greater amounts of levofloxacin loaded into it, such as in the case of F3 and F4. As mentioned earlier, this is due to the carboxyl group present in Na CMC, but this result is different from the findings of Buhus *et al*. (2009) wherein the quantity of CMC decreases drug loading into the hydrogel because of the repulsive interaction between CMC and chloramphenicol in which both are anionic in nature. Using one-way ANOVA, the acquired p = 0.427 which indicates that there is no significant difference between the means of the four groups indicating that drug loading is independent on polymer ratio of NaCMC and gelatin.

Hydrogel surface characteristics

Qualitative analysis was used to analyze the surface of the hydrogel wherein the functional groups such as O-H, C=O, and C-O were identified using FTIR indicating the formation of interconnected networks and efficient entrapment of levofloxacin inside cross-linked hydrogel at 1586.97/cm (Fig. 1).

As seen on SEM, the surface of uncrosslinked hydrogel was rough with irregularly-shaped pores, as shown in Fig. 2a-c. Unlike with cross-linked hydrogel on Fig. 2d-f, its intermolecular network chains were evident with 146 pores and an average pore area ratio of 61.27% due to added epichlorohydrin which stabilizes the structure and making it macroporous which is a good indication of a superabsorbent gel [13].

According to Ahmad *et al*. (2016), porosity is significant for drug loading due to its affinity with the aqueous medium causing the hydrogel to swell and this is evident on Fig. 2g-i in which the pores of loaded hydrogel have closed as it swelled due to diffusion of levofloxacin solution into the cross-linked hydrogel with 31 pores and average pore area ratio of 10.29%.

***In vitro* diffusional models and kinetics**

F3 and F4 hydrogels were selected due to its swelling property and drug loading percentage. As shown in Fig. 3, Oftaquix® was immediately released

Table 2: Physical characteristics of levofloxacin hydrogels

Formulation code	Average			
	Weight (g)	Thickness (mm)	Percentage swelling	EWC
F1	0.11	0.77	−23.62	−319.12
F2	0.28	0.70	1111.45	341.27
F3	0.44	0.48	1410.66	177.59
F4	0.66	0.35	1626.82	348.01

EWC: Equilibrium water content

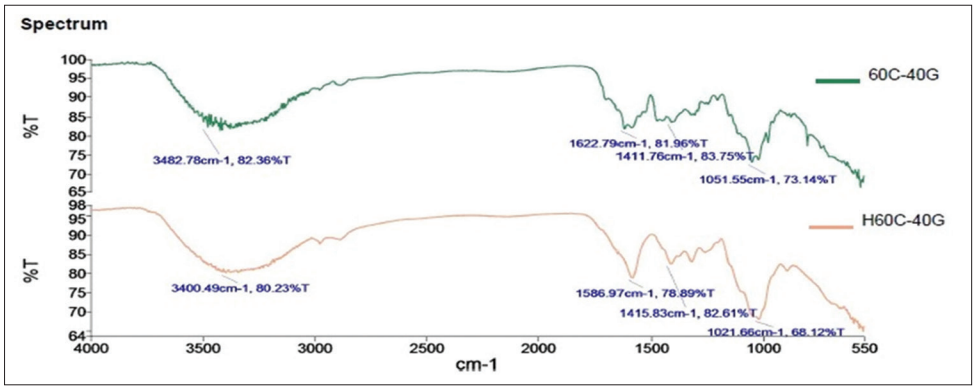


Fig. 1: Fourier-transform infrared spectra of crosslinked (60C:40G) and loaded hydrogels (H60C:40G)

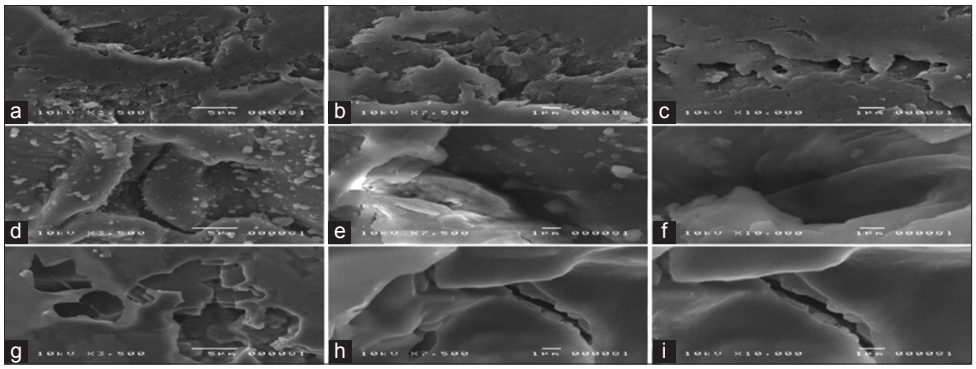


Fig. 2: Scanning electron microscopy images of uncrosslinked (a-c), crosslinked (d-f), and loaded hydrogels (g-i)

in simulated tear with higher drug concentration during the first 2 h but became constant on the 6th h. Meanwhile, loaded hydrogels were slowly released due to its viscosity, which took an hour before its concentration in the dissolution medium has increased. This can be explained by the findings of Hoosain *et al*. (2014) in which controlled swelling and dissolution are the two known mechanisms of how the drug is released from the hydrophilic matrix system. When a porous hydrogel is placed in an aqueous dissolution medium, the available pores allow diffusion of the drug within the surface of the matrix. It further allows water entry and closes when filled with the dissolution medium. The gellification of the system due to swelling leads to the dissolution of the polymer causing drug to be released. In addition, swelling rate and drug release at the time (t) are inversely proportional to each other. It means increased in swelling reduces the drug release rate because the drug molecule has to traverse the gel layer before it is released into the dissolution medium.

Table 3 suggests Higuchi as the kinetic model for loaded hydrogels with R = 0.991 (F3) and 0.994 (F4). As explained by Hoosain *et al*. (2016), drug diffusion from the polymeric network in Higuchi kinetics is decreasing when the distance for diffusion is increasing. It means the increase in distance will reduce the concentration of diffused levofloxacin in the STF. To explain the effect of swelling on matrix on hydration, Korsmeyer–Peppas exponent values were used [9] in which both hydrogels were described as fine films under super Case II transport wherein the classified release mechanism of F4 is anomalous transport (n = 0.951), while the drug release rate of F3 (n = 1.311) is controlled by hydrogel swelling in the dissolution medium and is time-independent with zero-order kinetics.

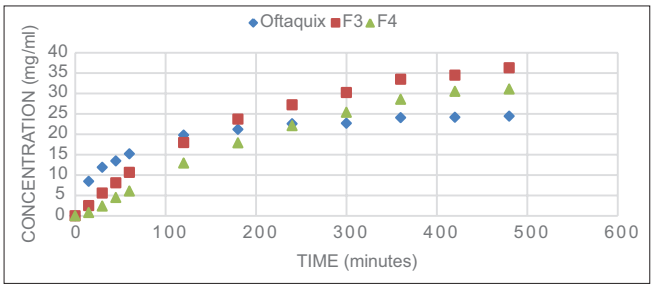


Fig. 3: Comparison of the *in vitro* drug release profile of levofloxacin from F3, F4, and 0.5% levofloxacin eye drops (Oftaquix®)

The mean differences of drug released per time were analyzed using two-way ANOVA in which the acquired p = 4.6044×10^{-8} indicating a significant difference; nevertheless, the drug release rate of each treatment group is still not significant from each other (p = 0.623).

In vitro bactericidal activities

Unloaded hydrogels did not exhibit any antibacterial activities against *P. aeruginosa* and *S. aureus.* However, if treated with loaded hydrogels, these common ocular pathogens were sensitive in which F3 has greater inhibitory zone of 31.68 and 37.05 mm, respectively. Using one-way ANOVA to the mean difference of Oftaquix® and F3 against *P. aeruginosa*, it was found that there is no significant difference (p = 0.897) on their antimicrobial property except for F4 with acquired p = 0.034. The result for *S. aureus* is also the same with no significant difference with F3 (p = 0.991) and F4 (p = 0.859).

Ocular irritancy profile

F3 was considered the optimized hydrogel among the four groups and instilled into the cul-de-sac of New Zealand rabbits. No visible signs of ocular damage had been observed within the 3-day treatment. Hence, the optimized levofloxacin hydrogel is non-irritating and safe as a treatment for ocular infections.

Stability profile of optimized levofloxacin hydrogel

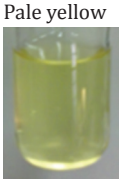
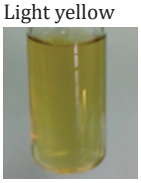
Three batches of 50 g optimized levofloxacin hydrogel (OLH) were prepared for accelerated stability studies for 3 months at 40 ± 2°C and 75 ± 5% relative humidity. As shown in Table 4, the OLH was pale yellow in color at 0 month. It is homogenous and nongritty in texture with an average pH of 3.94 and spreadability value of 0.430. Its viscosity made the hydrogel not flowable, but it gelled immediately with STF that last for more than 1 h.

After its 1st month inside the stability oven, the OLH still remained homogenous and nongritty in texture. However, due to its storage

Table 3: Kinetic model of OLH and Oftaquix®

Formulation code	R				Korsmeyer-Peppa	
	Zero-order	First-order	Higuchi	Hixson-Crowell	R	n
F4	0.9534	0.7248	0.9938	0.8358	0.9692	0.9514
F3	0.9307	0.7406	0.9905	0.8236	0.9754	1.311
Oftaquix	0.7993	0.7072	0.9168	0.7409	0.9620	3.2485

Table 4: Stability profile of OLH

Parameters	0 month	1st month	2nd month	3rd month
Visual appearance	Pale yellow	Light yellow	Yellow	Yellow orange
				
	Homogenous Nongritty	Homogenous Nongritty	Homogenous Nongritty	Homogenous Nongritty
pH	3.94±0.01	4.07±0.01	4.13±0.01	4.18±0.01
Viscosity (cp)	11.867±0.03	10.643±0.04	9.958±0.02	9.043±0.09
Spreadability ($\frac{g \times cm}{s}$)	0.430±0.08	0.601±0.07	0.376±0.06	0.399±0.10
Gelling capacity	+++	++	+	+

+: The vehicle is in liquid form which gels slowly and remains in less than an hour, ++: The vehicle is a liquid-gel, flows readily, and shows immediate gelation that remains for an hour, +++: The vehicle is a highly viscous gel, flows less readily and shows immediate gelation that remains for more than an hour

condition, its color turned into light yellow, pH increased to 4.07, while its viscosity decreased to 10.643 cp, making it liquid-gel in appearance that flowed less readily. Changes have still been observed for the next 2 months in which OLH at 3rd month was now yellow-orange in color, pH 4.18, and less viscous compared to 0 month in which the gel rapidly dissolved in STF. Loss of viscosity on storage is a common limitation of natural polymer especially when exposed at extreme temperature, but this can be improved by chemical modifications such as crosslinking, blending, and formation of interpenetrating polymer networks [10]. Changes in the pH and color of OLH are possibly an initial indication of levofloxacin degradation because quinolones are light sensitive and undergo photodegradation by first-order kinetics but this can be hindered by increasing the solvent viscosity, using of organic solvents, or formulating liquid preparations within pH 5–7 because degradation is greater in aqueous medium and higher pH due to formed cationic, dipolar, and anionic species caused by pH changes [14]. As observed, the viscosity of OLH is still acceptable for sustained-release ophthalmic preparations, and it decreases further degradation of levofloxacin at extreme storage conditions.

In addition, a significant increase in drug concentration of the OLH every month was observed [Table 5] in which the absorbance of hydrogel at 3rd month was higher than at 0 month as an effect of increased temperature, changes in pH, and presence of unreacted epichlorohydrin which aid in the continued swelling and drug loading of the polymer.

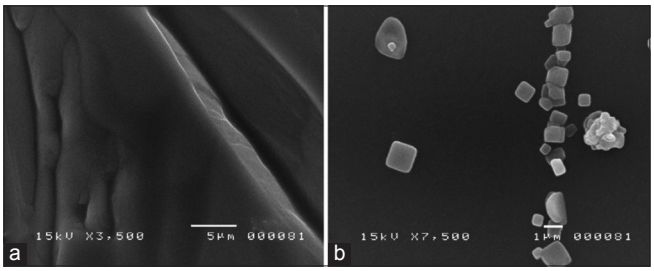


Fig. 4: Scanning electron microscopy image of OLH at 3rd month

According to Budianto *et al.* (2015), these ionic interactions are due to dissociation of hydrogen bonds in gelatin with water molecules present inside the hydrogel matrix.

At ×3500 magnification, as shown in Fig. 4a, the in-between spaces of OLH at 3rd month were now shallow compared to its SEM image at 0 month in which most of the pores are deep and irregularly-shaped. The tightening of the pores was due to continued entrapment of unloaded levofloxacin into the hydrogel. Crystals were also observed on its surfaces.

Fig. 5 indicates that the OLH at 3rd month was very well hydrated as shown by its endothermic peak at 79.53°C. It is because hydrogel films made from cellulose are vulnerable to moisture absorption [15] especially polymers containing high CMC content due to its high water vapor affinity which allows diffusion of water vapor molecules into the film causing it to swell greatly [16]. In addition, two sharp peaks at 223.20 and 243.57°C were observed, and this is due to crystallization, which was similarly observed on SEM image at ×3500 magnification. Increase in film crystallinity and high water vapor permeability decreases the amorphous properties of hydrogel film [17] leading to higher glass transition as evident by a shift to a higher temperature. It is an indication of strong hydrogen bonds due to continued crosslinking [10] caused by changes in temperature and pH.

There was no evident changes in the FT-IR spectra of OLH at 0 and 3rd month [Fig. 6]. It was still the same indicating the presence of O-H broad peaks at 3371.80/cm and 3388.87/cm, C=O stretch at 1575.65/cm, and C-O bond at 1009.44/cm and 1030.69/cm.

Antimicrobial activity

The three batches of the OLH have retained their antibacterial activities against *P. aeruginosa* and *S. aureus* with mean zones of inhibition of 32.64 mm [Table 6] and 40.22 mm [Table 7], respectively.. These indicate the sensitivity of these common ocular pathogens to the optimized hydrogel. However, Oftquix® had greater inhibitory zone for these two ocular pathogens with an average diameter of 37.24 and 40.63 mm, respectively.

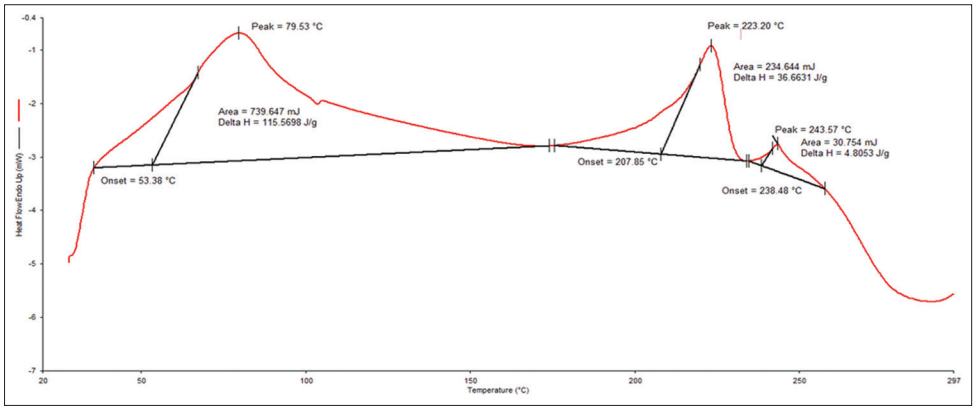


Fig. 5: Differential scanning calorimetry thermogram of OLH at 3rd month

Table 5: High performance liquid chromatography analysis of OLH

Stability period (40±2°C, 75±5% RH)	0 month	1st month	Percentage difference	2nd month	Percentage difference	3rd month	Percentage difference
Batch 1 (concentration in mg)	4.959±0.014	4.971±0.010	0.24	4.975±0.002	0.08	4.975±0.007	0
Batch 2 (concentration in mg)	4.964±0.005	4.972±0.006	0.16	4.973±0.007	0.02	4.973±0.001	0
Batch 3 (concentration in mg)	4.967±0.009	4.968±0.004	0.02	4.972±0.005	0.08	4.978±0.004	0

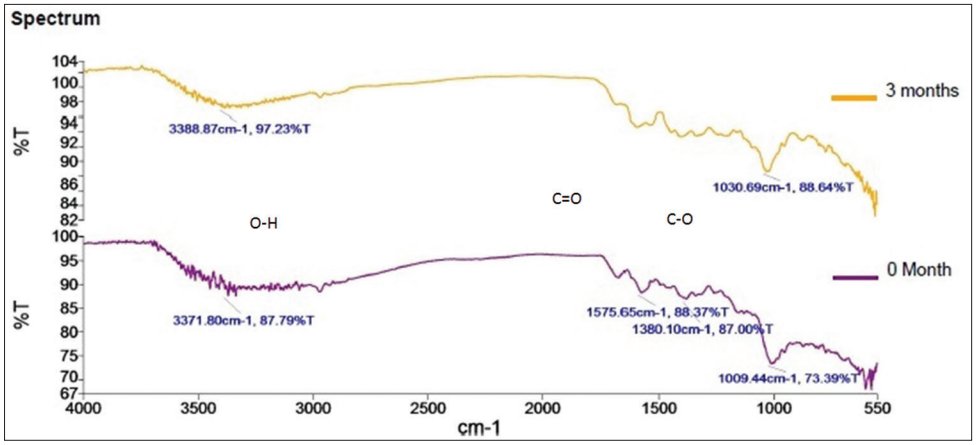


Fig. 6: Fourier-transform infrared spectra of OLH at 0 and 3rd months

Table 6: Antimicrobial activity of OLH against *Pseudomonas aeruginosa*

Zone of inhibition (mm)					
Treatment	**0 month**	**1st month**	**2nd month**	**3rd month**	**Average**
Oftaquix	31.59±0.60	34.28±0.72	32.76±1.06	37.24±2.42	37.24
F3	29.49±0.62	33.50±0.50	32.10±0.98	35.46±1.23	32.64
SWFI	0	0	0	0	0

p=0.000; n: 3×3 batches. SWFI: Sterile water for injection

Table 7: Antimicrobial activity of OLH against *Staphylococcus aureus*

Zone of inhibition (mm)					
Treatment	**0 month**	**1st month**	**2nd month**	**3rd month**	**Average**
Oftaquix	39.30±2.47	42.03±0.88	42.51±1.07	38.67±0.84	40.63
F3	39.65±2.55	40.99±0.29	42.60±0.69	37.62±0.77	40.22
SWFI	0	0	0	0	0

p=0.000; n: 3×3 batches. SWFI: Sterile water for injection

On the other hand, the mean difference between Oftaquix® and OLH against *P. aeruginosa* and *S. aureus* was statistically significant from each other (p = 0.000) using two-tailed paired sample *t*-test indicating that the antimicrobial activity of Oftaquix® is still superior to OLH.

Finally, data analysis using linear regression demonstrates that the optimized levofloxacin hydrogel follows zero-order kinetics with a half-life of 689 months and a shelf-life of 138 months when stored at 40°C.

CONCLUSION

The optimized levofloxacin hydrogel was stable for 3 months at 40°C. Its permeability, release rate and *in vitro* antibacterial activity were enhanced by formulating it into cross-linked hydrogel. In addition, it was non-irritating and can be used as an alternative for conventional 0.5% levofloxacin ophthalmic solution. Further studies should investigate its long-term stability and may initiate cost-analytical studies to determine its feasibility for commercialization.

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