RESEARCH PAPER

Qbd-Enabled Optimization and Analytical Characterization of Sitagliptin Loaded Bioadhesive Ocular Films for Enhanced Anti-Inflammatory Management

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ABSTRACT

Background: Ocular drug delivery poses major challenges owing to rapid precorneal elimination, low bioavailability (less than 5%), and drug residence time problems related to existing formulations such as eye drops and eye ointment. Sitagliptin (SIT) is an anti-inflammatory DPP-4 enzyme inhibitor and can be considered a promising drug for ocular use. This work was focused on the formulation and optimization of SIT-loaded ocular films (SITOFs).

Methodology: Hydroxypropyl Methylcellulose, HPMC K4M, Methyl Cellulose, MC, and Polyethylene Glycol was utilized as film-forming polymers and PEG-400 as a plasticizer in the solvent casting process to create SITOFs. 17 formulations of standard chemistry were optimized using the Box-Behnken design based on three independent factors. FTIR and DSC were utilized for preformulation compatibility studies. Films were characterized by their physiochemical properties, in vitro corneal permeation, antimicrobial activity, in vitro drug release kinetics, antioxidant activity, and in vivo ocular irritation and anti-inflammatory activity.

Findings: FTIR and DSC analysis proved the compatibility between the drug and the polymer. Films possessed adequate physicochemical parameters such as uniform thickness (59-200 µm), uniform weight (26-110 mg), drug content (69.52-101.97%), and swelling index (80.2-94.21%). The improved formulation SITOF-1 followed Higuchi kinetics of diffusion with non-Fickian transport ($n < 0.5$) and had set drug release qualities (around 85% drug release after 240 min). The drug's effective transcorneal permeability from the formulation was validated by an ex vivo permeation investigation. In addition to their dose-dependent ability to scavenge DPPH radicals, SITOFs demonstrated encouraging antibacterial effectiveness against Pseudomonas aeruginosa (ATCC 25857) and Staphylococcus aureus (ATCC 25923). In vivo research revealed that there was no irritation.

Conclusion: SITOFs developed have proved to be a safe, efficacious, and compliant ocular drug delivery system and thus hold promising prospects for the treatment of ocular inflammation and infections. The results validate the repurposing approach for sitagliptin for ophthalmic use.

Keywords: Ocular drug delivery, Sitagliptin, Ocular films, Box-Behnken design, optimized formulation, controlled drug release, Repurposing

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INTRODUCTION

One of the most difficult topics in pharmaceutics is that of ocular drug delivery due to the specialized anatomy and physiology of the eye. The various mechanisms of precorneal elimination, which include tear turnover, nasolacrimal draining, eyelid movement, and the cornea's poor permeability, together result in less than 5% bioavailability of conventionally applied drugs[1,2]. The commonly used dosage forms such as eyedrops and ointments, while extensively applied in practice, possess inherent drawbacks such as fast precorneal elimination, blurred vision, poor patient compliance, and frequent drug administration[3,4]. This has led to extensive research on more sophisticated drug delivery systems; ocular drug delivery systems (ODDS); that ensure adequate residence time of the drug on the ocular surface, sustained drug delivery, and increased penetration of drugs through the cornea.

Ocular films of polymers, referred to as ocular inserts, represent a new promising drug delivery system for

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sustained ocular drug delivery. The ocular films have the following benefits over traditional systems: precorneal retention, low systemic absorption, directed drug delivery, and good patient compliance due to reduced frequency of dosing[5,6]. Some polymers, among which Hydroxypropyl Methylcellulose (HPMC K4M), Methyl Cellulose (MC), represent the well-characterized, biocompatible, and biodegradable materials with good film forming, bioadhesive and hydrated controlled release properties[7,8]. Addition of Polyethylene Glycol (PEG-400) as a plasticizer is beneficial for improved flexibility and permeability of drug through films.

In addition to its anti-hyperglycemic action, sitagliptin (SIT), a powerful and selective inhibitor of the DPP-4 enzyme used to treat type 2 diabetes, has anti-inflammatory properties[9]. SIT reduces inflammation by inhibiting pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, controlling macrophage activity, and stimulating T-regulatory cells[10]. Inflammation is considered one of the primary pathophysiological mechanisms involved in eye diseases such as conjunctivitis, keratitis, and uveitis. Thus, SIT can be regarded as a promising drug for the repurposing into the field of ophthalmology as an anti-inflammatory agent due to its proven safety profile and anti-inflammatory mechanism of action, especially taking into consideration the risk of increased intraocular pressure and corneal toxicity associated with use of glucocorticoids and NSAIDs.

The ICH Q8(R2) guidelines' Quality by Design (QbD) principles offer an organized, methodical framework for the creation and optimization of pharmaceutical formulations[11]. The Box Behnken Design (BBD), response surface methodology that enables the efficient evaluation of several formulation parameters and their interactions with less trial runs, makes it easier to find the optimal formulation compositions[12,13]. Despite extensive literature on ocular film formulations, the application of QbD-guided BBD optimization to sitagliptin-loaded ocular films with a comprehensive evaluation of anti-inflammatory, antimicrobial, and antioxidant efficacy has not been previously reported.

With a thorough assessment of physicochemical characteristics, drug release kinetics, ex vivo corneal penetration, antimicrobial activity, antioxidant capacity, in vivo irritation, and anti-inflammatory efficacy, the current study sought to develop and optimize SITOFs using HPMC K4M, MC, and PEG-400 using a BBD-guided QbD approach.

MATERIALS & METHODOLOGY

Materials

Microlabs Ltd., Bengaluru, India, provided a free sample of SIT phosphate monohydrate. We bought MC, HPMC K4M, and PEG-400 from Smart Biolab in India. Simulated tear fluid (STF, pH 7.4) was prepared using the standard procedure. The Muller-Hinton agar and broth were purchased from HiMedia Laboratories in Mumbai, India. All of the remaining chemicals and reagents were of analytical quality.

Pre-formulation Studies

Binary physical mixtures consisting of SIT were prepared with each excipient (HPMC K4M, MC, and PEG-400) in a 1:1 ratio (w/w) in order to perform pre-formulation compatibility testing. Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu IRTracer-100, Japan) within the range of 4000–400 cm^{-1} as well as Differential Scanning Calorimetry (DSC; Shimadzu DSC-60, Japan) at a standard thermal rate of 10 °C/min under nitrogen purge (50 mL/min) were used for analyzing the mixtures. Drug-excipient interaction was thought to be indicated by any changes or removal of distinctive peaks.

Formulation of Sitagliptin Ocular Films

The solvent casting method was used to create SITOFs. PEG-400 was incorporated as a plasticizer after HPMC K4M and MC were individually dissolved in STF (pH 7.4) with a magnetic stirrer. The polymer solution was continuously stirred to dissolve SIT (0.1% w/v) until a homogenous solution was achieved. After degassing, the mixture was transferred into glass Petri dishes (diameter: 90 mm) that had been previously treated. After that, the plates were dried in a hot-air oven at 40 °C for 24 hr. A stainless-steel punch was used to cut 6 mm-diameter ocular inserts from the cast films after they had dried. According to the Box-Behnken Design matrix, seventeen formulations were created (Table 1).

Box-Behnken Design (BBD) Optimization

To improve the formulation, a three-factor, three-level BBD was used with Design Expert® software (version 13, Stat-Ease Inc., Minneapolis, USA). MC concentration (B: 5-30 mg), HPMC K4M concentration (A: 20-80 mg) and PEG-400 volume (C: 0.1-0.3 mL) were independent factors. Entrapment efficiency (EE%) and cumulative drug release (% CDR at 240 min) were the dependent responses. To reduce error during the experiment, a total of 17 experimental runs with five center points were produced. For every response, polynomial regression equations were created, and the desired function method was used to determine the best formulation.

Table 1: Composition of sitagliptin ocular films (SITOFs) as per Box-Behnken Design

Formulation	*HPMC K4M (milligram)*	*PEG-400 (miliLitre)*	*MC (milligram)*

SITOF-1	20	0.2	5
SITOF-2	20	0.1	17.5
SITOF-3	50	0.3	30
SITOF-4	20	0.2	30
SITOF-5	50	0.2	17.5
SITOF-6	20	0.3	17.5
SITOF-7	50	0.2	17.5
SITOF-8	80	0.3	17.5
SITOF-9	80	0.1	17.5
SITOF-10	50	0.1	5
SITOF-11	50	0.2	17.5
SITOF-12	50	0.1	30
SITOF-13	50	0.2	17.5
SITOF-14	50	0.3	5
SITOF-15	50	0.2	17.5
SITOF-16	80	0.2	5
SITOF-17	80	0.2	30

Physicochemical Evaluation

The following physicochemical properties were assessed for each of the 17 formulations.USA). A digital micrometer (Mitutoyo, Japan) was used to determine the film thickness at three distinct locations, and the mean ± SD was computed. Three randomly chosen inserts from each batch were weighed on an analytical balance (Shimadzu AUX220, Japan) to assess weight consistency. Drug content was assessed spectrophotometrically (UV-1800, Shimadzu, Japan) at λmax 265 nm after complete dissolution of the insert in STF (pH 7.4). The swelling index (SI) was calculated from pre- and post-hydration weights after immersion in STF at 37 °C. Moisture content and moisture uptake were also determined gravimetrically.

In Vitro Drug Release Studies

In vitro SIT release was performed using a modified Franz diffusion cell assembly and a dialysis membrane (MWCO 12,000 Da, Sigma-Aldrich) as the barrier between the donor and receptor compartments. STF, with a pH of 7.4, was kept at 37 ± 1 °C the receptor compartment while being thoroughly agitated at 20 rpm. At predetermined intervals (0, 30, 60, 90, 120, 180, and 240 minutes), samples (1 mL) were taken out of the dissolving solution and replaced with new STF. The amount of SIT in the dissolving liquid was determined via measurement of absorbance at 265 nm. The drug release rate was plotted and evaluated using Higuchi, Korsmeyer-Peppas, zero-order, and first-order equations.

Ex Vivo Corneal Permeation Studies

For the ex vivo corneal permeation studies, freshly enucleated goat corneas from the local butcher shop were procured within two hours after slaughter. The cornea was positioned in between the donor and receptor compartments of the Franz diffusion cell, with the epithelium the donor compartment. The receptor compartment was reconstituted with STF (pH 7.4) and continuously swirled at 20 rpm at 37 ± 1 °C, while the donor compartment was given an ocular insert with 0.7 µL of STF to simulate tear volume. Occasionally, samples were gathered and subjected to spectrophotometric analysis at 265 nm. The steady state flow (Jss), apparent permeability (Papp), and permeability coefficient (Kp) were calculated.

Antimicrobial Activity

The antibacterial activity of SITOFs was evaluated against Pseudomonas aeruginosa (ATCC 25857) and Staphylococcus aureus (ATCC 25923) employing the disc diffusion assay on Muller-Hinton agar medium. Standard bacterial suspensions were made by meeting the 0.5 McFarland turbidity standard (~1.5 x 10^2 CFU/mL). The inoculated plates were treated with SITOFs, moxifloxacin discs (positive control), and placebo film (negative control). The zones of inhibition (ZOI, mm) were measured using digital calipers after a 24-hour incubation period at 37 ± 1 °C. To find a significant difference ($p<0.05$), the Student's t-test was employed.

Antioxidant Activity (DPPH Assay)

The ability of SITOFs to scavenge free radicals was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The working solution's absorbance was modified to roughly 0.936 at 517 nm following the creation of the DPPH stock solution (24 mg/100 mL of methanol). The range of concentrations utilized was 1–40 µg/mL. Equation (1) was used to calculate the percentage of radical scavenging activity.

% Radical scavenging activity = [(Ac – As) / Ac] × 100 (Eq. 1); (Ac = absorbance of DPPH control; As = absorbance of test sample).

Accelerated Stability Studies

The improved composition, SITOF-1, underwent accelerated stability investigations for six months at 25 °C ± 2 °C/60% RH ± 5% RH in a temperature-humidity stability chamber (Hally Instruments, Mumbai, India) in compliance with ICH Q1A(R2) standards. Every month, the medication concentration, surface pH, thickness, clarity, and physical look of the samples were examined.

In Vivo Studies

The Institutional Animal Ethics Committee of Centurion University of Technology and Management, Bhubaneswar, India, granted approval for all animal experiment procedures (Approval No. CUTM/IAEC/45), and the CPCSEA and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were closely followed.

Ocular Irritation Test: The Draize test was used to conduct a biocompatibility research in six albino rabbits weighing between 2.1 and 2.5 kg. The left eye's conjunctival sac was filled with SITOF-1, which was optimized, whereas the right eye was used as a control group and was given regular saline. Both eyes were examined for conjunctival redness, chemosis, and discharge at 0, 12, 24, and 48 hours.

In Vivo Anti-Inflammatory Test: An immediate ocular inflammatory condition was produced when 10 µL of clove oil was applied to the lower conjunctival sac of the left eye of healthy albino male rabbits (1.5–2.5 kg, n = 6 for each group). Three rabbit groups were chosen: Normal saline was given to Group I (positive control); Group III (test) was given SITOF-1 after inflammation was induced, while Group II (standard) was given prednisolone acetate 1% ophthalmic suspension (Pred Forte®). The degree of ocular inflammation was measured using the modified Draize score at 0, 1, 3, 6, 12, 24, 48, and 72 hours after the onset of ocular inflammation using corneal opacity, conjunctival redness, edema, and discharge. The statistics were compared using a one-way ANOVA test and Tukey's post-hoc test ($p<0.05$).

Greenness Assessment

AGREE (Analytical GREEnness) and GAPI (Green Analytical Procedure Index) were used to evaluate the analytical approach's environmental sustainability. While GAPI is displayed as a collection of color-coded pictograms that correspond to different environmental impacts, AGREE scores range from 0 (not green at all) to 1 (maximum greening).

RESULTS AND DISCUSSIONS

Pre-formulation Compatibility Studies

According to data from the literature, sitagliptin's FTIR spectra showed clear peaks at 3295 cm^{-1} (N-H stretching), 1710 cm^{-1} (C=O stretching of amide), 1643 cm^{-1} (C=N stretching), and 1175 cm^{-1} (C-F stretching)[14]. For physical blends of SIT with HPMC K4M, MC, and PEG-400, all distinct peaks associated with sitagliptin were observed without any shift, thereby suggesting no chemical reaction of the drug with other constituents (Fig 1 (a)). The DSC plot of pure SIT revealed an intense endotherm at 256.8 °C related to its melting temperature, which was unchanged for all physical mixtures, confirming the compatibility and chemical stability of the drug in the polymer matrix (Fig 1 (b)).

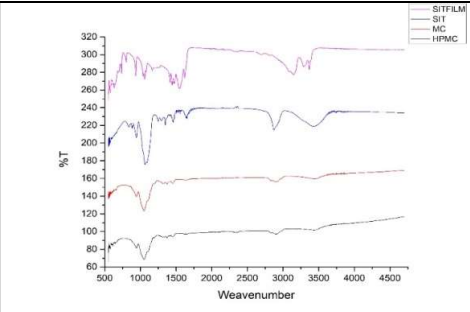


Figure 1 (a):FTIR spectra of pure sitagliptin and physical mixtures with HPMC K4M, MC, and PEG-400 showing compatibility

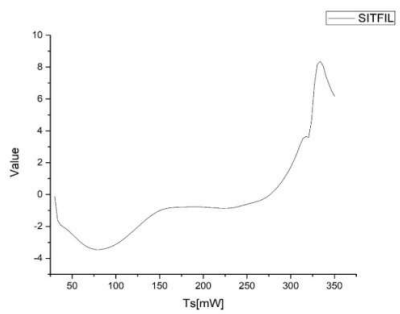


Figure 1 (b): DSC thermograms of pure sitagliptin and drug–polymer physical mixtures indicating absence of drug–excipient interactions

Physicochemical Characterization of SITOFs

All 17 formulations were successfully prepared as flexible films having uniform surfaces. Physiochemical data for all the formulations is provided in Table 2. The thickness of films varied from 59 ± 0.57 µm (SITOF-1) to 200 ± 0.57 µm (SITOF-17), where increase in the thickness was due to the increase in total amount of polymer. Small standard deviation values indicated uniform distribution of thickness among all the formulations. Weight uniformity was between 26 ± 0.57 mg (SITOF-1) and 110 ± 0.57 mg (SITOF-17), being dependent on the amount of polymer in directly proportional manner with consistently low variability.

The drug content varied from 69.52 ± 0.32% (SITOF-9) to 101.97 ± 0.46% (SITOF-4). Center point replicates (SITOF-5, 7, 11, 13, 15) had drug content value around 99-100%, thereby proving that the procedure used for preparation of the formulation was reliable and reproducible. The Swelling Index, a crucial indicator of ocular residence time and drug release properties, ranged from 80.23 ± 1.06% (SITOF-12) to 94.21 ± 0.65% (SITOF-17). Higher hydrophilic polymer containing formulations had high swelling index values, implying the higher moisture absorption capability of the formulations which is an ideal characteristic for sustained drug release.

Table 2: Physicochemical properties of sitagliptin ocular films (SITOFs; n = 3, mean ± SD)

Formulation	*Weight (mg)*	*Thickness (µm)*	*Drug Content (%)*	*Swelling Index (%)*
SITOF-1	26 ± 0.57	59 ± 0.57	96.97 ± 1.48	90.27 ± 0.60
SITOF-2	37 ± 0.58	83 ± 1.00	84.42 ± 0.63	81.90 ± 0.55
SITOF-3	81 ± 0.56	147 ± 1.52	98.45 ± 0.43	86.00 ± 0.14
SITOF-4	49 ± 1.52	98 ± 1.00	101.97 ± 0.46	83.60 ± 0.49
SITOF-5	67 ± 1.23	121 ± 1.52	99.27 ± 0.49	84.89 ± 0.73
SITOF-6	38 ± 1.00	87 ± 1.52	91.47 ± 0.55	84.44 ± 0.81
SITOF-7	67 ± 1.52	122 ± 1.52	99.22 ± 0.20	84.96 ± 0.65
SITOF-8	98 ± 1.00	182 ± 1.00	79.35 ± 0.62	92.25 ± 0.19
SITOF-9	97 ± 1.52	169 ± 0.57	69.52 ± 0.32	88.45 ± 1.00
SITOF-10	55 ± 1.53	84 ± 0.57	77.25 ± 0.64	85.36 ± 0.80
SITOF-11	67 ± 1.51	120 ± 1.00	99.36 ± 0.52	84.42 ± 0.92
SITOF-12	80 ± 1.00	132 ± 1.00	94.32 ± 0.54	80.23 ± 1.06
SITOF-13	67 ± 1.52	120 ± 1.52	99.31 ± 0.45	84.17 ± 0.92
SITOF-14	55 ± 1.53	85 ± 1.00	90.15 ± 1.05	89.44 ± 1.10
SITOF-15	67 ± 1.00	121 ± 1.00	99.33 ± 0.50	84.95 ± 0.33
SITOF-16	85 ± 1.00	137 ± 1.15	75.72 ± 0.42	93.86 ± 1.14
SITOF-17	110 ± 0.57	200 ± 0.57	95.69 ± 0.44	94.21 ± 0.65

Box-Behnken Design Analysis and Statistical Optimization

The effect of concentration of HPMC K4M (A), MC (B), and PEG-400 (C) on entrapment efficiency (EE) and % CDR was studied using BBD. The quadratic model achieved strong significance in every response ($p < 0.0001$), according to the ANOVA output (Table 3). All independent factors had highly significant effect on EE and % CDR. The interaction factor AB (HPMC-MC) had the highest influence among the interaction factors, whereas PEG-400 (C) had significant effect on film thickness and drug release.

In coded factor form, the final regression equations were obtained as;

$$EE\ (\%) = 81.06 + 8.99A + 6.29B - 3.94C + 11.87AB - 1.04AC - 2.35BC - 18.15A^2 - 2.74B^2 - 8.37C^2$$

$$\%\ CDR = 59.61 + 0.4038A - 11.68B + 1.72C - 5.03AB + 8.72AC - 3.18BC + 8.06A^2 - 4.71B^2 + 5.60C^2$$

EE equation's negative quadratic term for A2 denotes an ideal polymer concentration over which entrapment efficiency decreases, most likely as a result of higher matrix density restricting drug incorporation. Greater MC concentration will slow down the drug release process through gel formation, as indicated by the negative value of B in the connection between MC concentration and drug release rate. The minimal criteria of 4 was exceeded by the values of Adequate Precision for EE (61.012) and % CDR (20.933). As a result, the model's signal-to-noise ratio was adequate. Model validity was assessed using the Normal Probability Plot, Residual vs. Run Order Plot, and Predicted vs. Actual Plot.

Table 3: ANOVA for quadratic model responses; Entrapment Efficiency (EE) and Cumulative Drug Release (% CDR)

Source	***SS (EE)***	***F (EE)***	***p (EE)***	***SS (CDR)***	***F (CDR)***	***p (CDR)***
Model	8428.87	5244.63	< 0.0001	22943.07	1614.90	< 0.0001
A-HPMC	7200.00	40320.00	< 0.0001	16290.13	10319.54	< 0.0001
B-MC	1225.13	6860.70	< 0.0001	5618.00	3558.91	< 0.0001
C-PEG	1.13	6.30	0.0404	136.13	86.23	< 0.0001
AB	1.00	5.60	0.0499	144.00	91.22	< 0.0001
AC	0.00	0.00	1.0000	20.25	12.83	0.0090
BC	0.25	1.40	0.2753	49.00	31.04	0.0008
A^2	0.07	0.37	0.5630	462.00	292.67	< 0.0001
B^2	0.59	3.32	0.1114	254.53	161.24	< 0.0001
C^2	0.59	3.32	0.1114	4.42	2.80	0.1380
Residual	1.25	—	—	11.05	—	—
Lack of Fit	1.25	—	0.1097	8.25	3.93	0.1097

SS = Sum of squares; F = F-value; p = probability value

In Vitro Drug Release Kinetics

Every formulation had a comparable drug release pattern that included two stages: the burst release stage, which occurred within 30 to 60 minutes after drug delivery, and the sustained release stage, which occurred after 30 to 60 minutes and lasted for up to 240 minutes. The hydrophilic polymeric matrix system's properties, which show surface erosion throughout the first stage and diffusion through the gel layer generated during the second stage, are responsible for the biphasic release profile[15]. Because of its high polymer content, SITOF-12 had the largest release (~98%) and SITOF-9 had the lowest release (~50–60%), whereas SITOF-1 had the best controlled drug release profile with no burst impact.

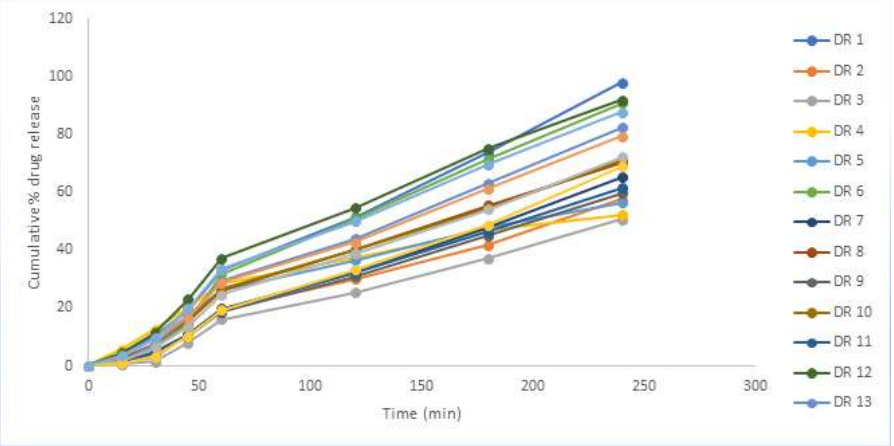


Figure 2: In vitro cumulative drug diffusion profiles of sitagliptin-loaded ocular films (SITOF-1 to SITOF-17) in simulated tear fluid (pH 7.4) over 240 min

Higuchi, Korsmeyer-Peppas, zero order, and first order kinetic models were found to fit the release kinetics quite effectively (Table 4). SITOF-1's match to the Higuchi model (R2 = 0.974) was the best of the four kinetic models, indicating the diffusion-controlled release from matrix system. The Korsmeyer-Peppas power index ($p<0.5$) of SITOF-1 indicated that the Fickian diffusion was the major release mechanism while there were some other contributions through non-Fickian diffusion in a few other film types[16,17].

Table 4: Release kinetic parameters of sitagliptin-loaded ocular films (SITOFs) fitted to Higuchi, zero-order, Korsmeyer–Peppas models, first-order kinetic model

Formulation	*Higuchi R^2*	*Zero-order R^2*	*Korsmeyer–Peppas R^2*	*First-order R^2*
SITOF-1	0.9741	0.9236	0.8098	0.9236
SITOF-2	0.8925	0.7915	0.8055	0.7915
SITOF-3	0.8935	0.7168	0.9657	0.7168
SITOF-4	0.8696	0.6724	0.9949	0.6724
SITOF-5	0.9007	0.7370	0.9791	0.7379
SITOF-6	0.7875	0.5727	0.983	0.5727
SITOF-7	0.8198	0.6206	0.9860	0.6206
SITOF-8	0.9595	0.85	0.8681	0.85
SITOF-9	0.8664	0.8701	0.6841	0.8701
SITOF-10	0.9469	0.9744	0.7123	0.9744
SITOF-11	0.9353	0.9545	0.673	0.9545
SITOF-12	0.9482	0.8858	0.7725	0.8858
SITOF-13	0.9403	0.849	0.8043	0.849
SITOF-14	0.9514	0.9628	0.7023	0.9628
SITOF-15	0.9822	0.9476	0.7932	0.9471
SITOF-16	0.8919	0.9598	0.6313	0.9598
SITOF-17	0.9744	0.9234	0.8101	0.9234

Optimization and Validation

According to the desirability function method, the optimized formulation was SITOF-1, with a value of desirability being 0.901. With a mean prediction error of less than 5%, the predicted values (weight: 26.21 mg; thickness: 57.69 µm; drug content: 97.74%; swelling index: 90.67%) closely matched experimental values (26 ± 0.57 mg; 59 ± 0.57 µm; 96.97 ± 1.48%; 90.27 ± 0.60%), demonstrating the robustness and dependability of the BBD optimization model (Figure 3 & table 5).

Table 5: Comparison of predicted and experimental responses for the optimized formulation SITOF-1

Parameter	Predicted Value	Experimental Value	% Prediction Error

Weight (mg)	26.21	26.00 ± 0.57	< 1%
Thickness (µm)	57.69	59.00 ± 0.57	2.3%
Drug Content (%)	97.74	96.97 ± 1.48	0.8%
Swelling Index (%)	90.67	90.27 ± 0.60	0.4%

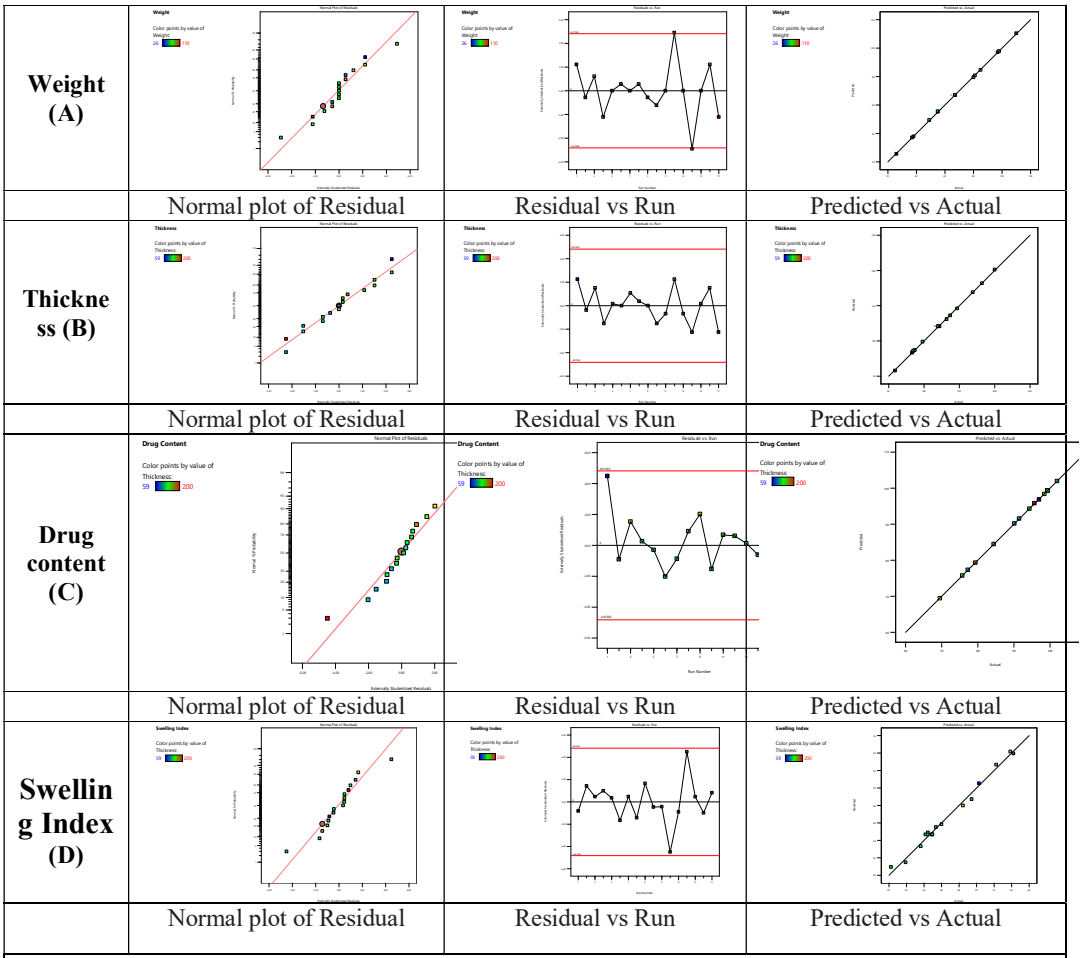


Figure 3: Diagnostic plots of response surface models showing normal probability plots, residual versus run plots, and predicted versus actual plots for (A) weight, (B) thickness, (C) drug content, and (D) swelling index responses.

Ex Vivo Corneal Permeation

Ex vivo corneal permeation of SIT from the improved SITOF-1 via recently removed goat cornea showed a permeability profile consistent with its in vitro release kinetics and consistent and prolonged drug transport across the corneal barrier. The drug permeated progressively through the corneal membrane, confirming the ability of SITOFs to overcome the corneal epithelial barrier. The reason behind efficient transcorneal drug delivery by this method can be the increased permeability due to hydration along with the bioadhesiveness of HPMC-MC matrix[18]. This indicates that SITOF-1 has the ability to deliver clinically effective amounts of SIT into the corneal tissues (Fig 4).

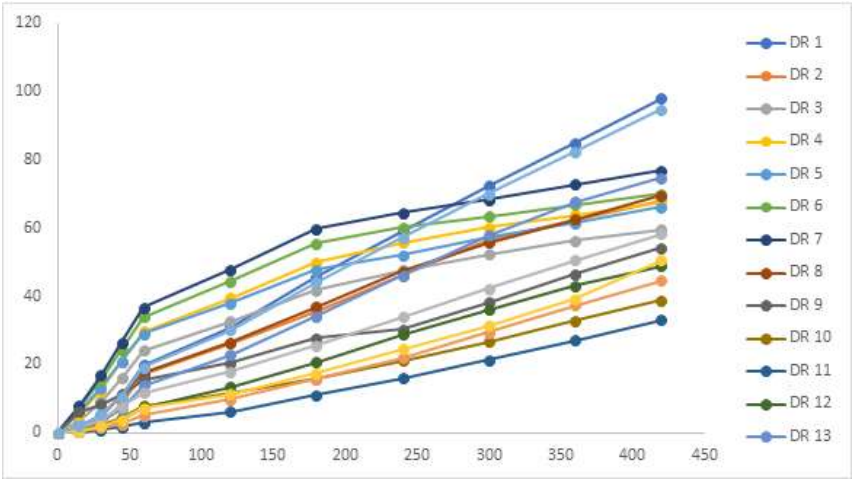


Figure 4: Ex vivo corneal permeation profiles of sitagliptin from ocular films (SITOF-1 to SITOF-17) across freshly excised goat cornea using Franz diffusion cells.

Antimicrobial Activity

Both Gram-negative (P. aeruginosa, ATCC 25857) & Gram-positive (S. aureus, ATCC 25923) bacteria were used to investigate the antimicrobial efficacy of SIT-loaded ocular films (Fig 5). Following a 24-hour incubation period at 37 °C, all zones of inhibition surrounding SIT-loaded films were detectable on both the bacterial plates. Placebo films (HPMC + MC + PEG-400) showed no inhibitory zones, indicating that the polymeric carrier itself has no antibacterial qualities. As anticipated, the moxifloxacin disc (positive control) displayed the largest zones of inhibition. SIT-loaded film-forming systems demonstrated statistically significant ($p < 0.05$) zones of inhibition for both bacteria, suggesting that SIT can be used as an antibacterial agent in ophthalmology[19].

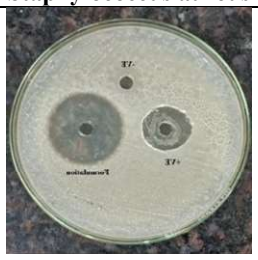
Staphylococcus aureus	Pseudomonas aeruginosa
	
a	B

Figure 5: Antimicrobial activity of sitagliptin-loaded ocular films against (A) *Staphylococcus aureus* (ATCC 25923) & (B) *Pseudomonas aeruginosa* (ATCC 25857) determined by agar diffusion assay.

Antioxidant Activity

The DPPH radical scavenging activity of SITOFs showed a dose-dependent increase across concentrations of 1–40 µg/mL. The optimized SITOF-1 demonstrated scavenging activity increasing from 6.45 ± 0.32% at 1 µg/mL to 64.89 ± 1.23% at 40 µg/mL, with an IC_{50} value of 31.78 ± 1.05 µM. This was significantly lower than IC_{50} values of individual polymer films (HPMC: 39.57 µM; MC: 50.78 µM; PEG: 34.49 µM), suggesting a synergistic antioxidant effect within the composite formulation. The ascorbic acid standard exhibited an IC_{50} of 18.95 ± 1.16 µM (Fig 6). These antioxidant properties may contribute to attenuating oxidative stress-mediated tissue damage in inflammatory ocular conditions.

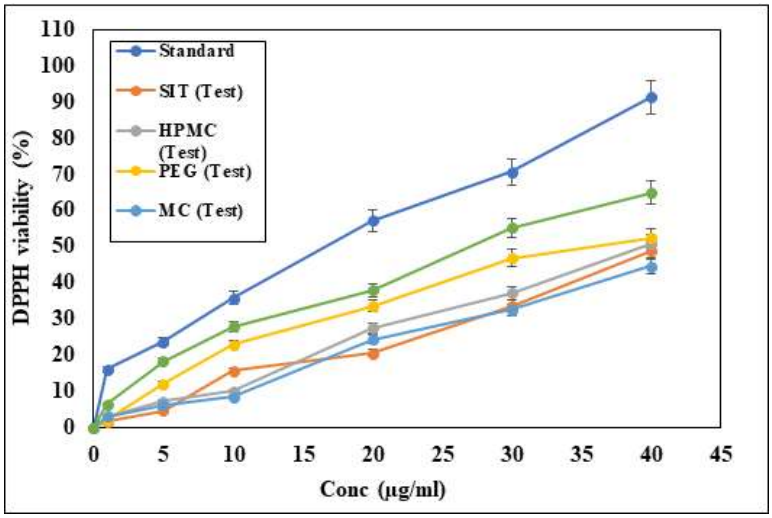


Figure 6: DPPH free radical scavenging activity of sitagliptin, individual polymers (HPMC, MC, PEG-400), and optimized SITOF formulation at different concentrations (1–40 µg/mL).

Accelerated Stability Studies

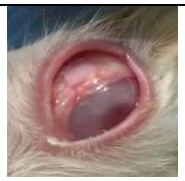
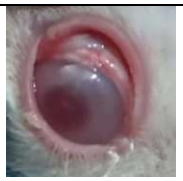
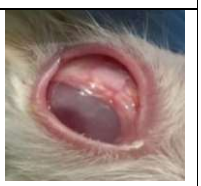
Over SITOF-1 outstanding stability under accelerated storage conditions (25 °C/60% RH). Surface pH (7.1-7.2), transparency (90.92-91.85%), Drug content (99.63 ± 0.66% to 99.73 ± 0.59%) and thickness (0.082-0.083 mm) did not vary significantly ($p > 0.05$) over the course of the investigation (Table 6). Visual examination verified that films maintained their smooth texture, clarity, and physical integrity without any indications of delamination, crystallization, or polymer degradation, indicating a sufficient shelf-life under the tested circumstances.

Table 6: Accelerated stability data for SITOF-1 at 25 °C ± 2 °C / 60% RH ± 5% RH over 6 months

Parameter	***0 Month***	***1 Month***	***3 Months***	***6 Months***
Drug Content (%)	99.63 ± 0.66	99.98 ± 0.68	98.98 ± 0.86	99.73 ± 0.59
Transparency (%)	91.85 ± 0.62	91.72 ± 0.75	90.92 ± 0.15	91.52 ± 0.69
Surface pH	7.1 ± 0.05	7.2 ± 0.02	7.1 ± 0.06	7.2 ± 0.05
Thickness (mm)	0.082 ± 0.004	0.083 ± 0.006	0.082 ± 0.005	0.083 ± 0.004
Visual Appearance	Clear, smooth	Clear, smooth	Clear, smooth	Clear, smooth

In Vivo Ocular Irritation Study

Draize test in albino rabbits revealed zero scores for conjunctival redness, chemosis, and discharge in all animals receiving SITOF-1 at all-time points (0, 12, 24, and 48 h). There were no signs of any irritation, swelling, or corneal opacity in the eyes throughout the study period. This shows that the developed formulation is non-irritant to the eyes as the materials used are biocompatible. This is because HPMC, MC, and PEG-400 are all ingredients found in the FDA's inactive ingredient database and the generally recognized as safe (GRAS) list (Fig 7).

Irritation	Day 0	Day 1	Day 2
Positive control			

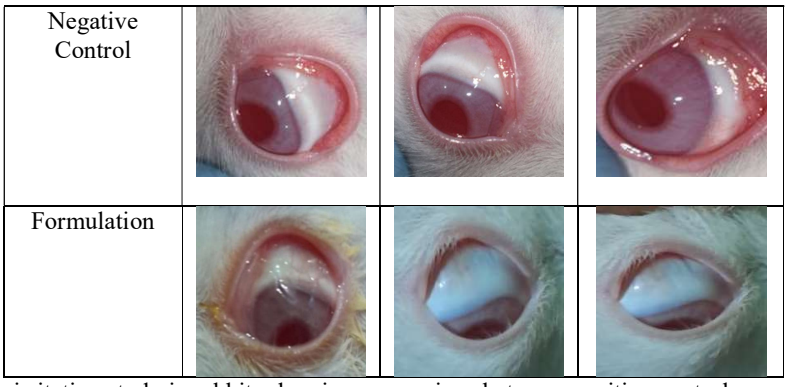


Figure 7: Ocular irritation study in rabbits showing comparison between positive control, negative control, and optimized SITOF formulation over 48 h.

In Vivo Anti-Inflammatory Activity

The incorporation of clove oil succeeded in inducing acute inflammation of the eyes in albino rabbits as confirmed by corneal opacity, reddening of the conjunctiva, chemosis, and secretion. The anti-inflammatory activity of SITOF-1 was measured for 72 hours using the modified Draize scale. The standard drug-treated group (prednisolone acetate 1%) showed relatively rapid onset of resolution within 1–3 hours, in line with the strong action of the corticosteroid. But SITOF-1 showed similar anti-inflammatory activity beyond 6 hours post-instillation, where there was continuous reduction of the inflammation score to reach 86.9% reduction in the SITOF-1 group and 80.8% in the prednisolone group at 72 hours. Inflammation score differences between SITOF-1 and the positive control group were significantly different ($p < 0.001$) at all time intervals. The contralateral eye, which is a healthy eye, had zero inflammation scores throughout the experiment (Fig 8). These results give in vivo evidence of the anti-inflammatory property of sitagliptin in the ocular film system probably via the DPP-4 enzyme inhibiting action and subsequent suppression of pro-inflammatory cytokines[20-21].

Greenness Assessment

The greenness assessment through AGREE gave an AGREE value of 0.87, which indicates strong compliance to the 12 principles of Green Analytical Chemistry. The results were clearly indicated by the radar plot showing strong green scores for all areas especially the solvent hazard reduction, energy use and waste production (Figure 9). The pictogram from the GAPI software confirms that the method is green in all aspects such as the solvent used, detection method and sample handling. The minor yellow designation in the bioanalytical sample preparation steps reflects the use of membrane filtration, an unavoidable step in ex vivo studies, which does not substantially compromise overall greenness.

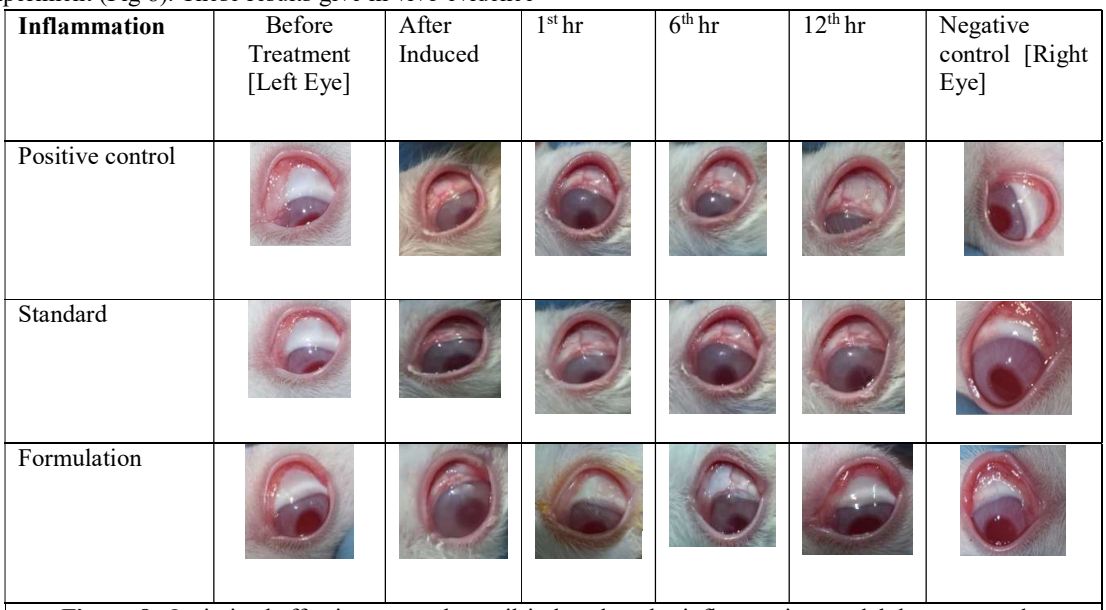


Figure 8: Optimized effectiveness a clove oil-induced ocular inflammation model demonstrated a progressive decrease in inflammation when compared to controls and regular prednisolone treatment.

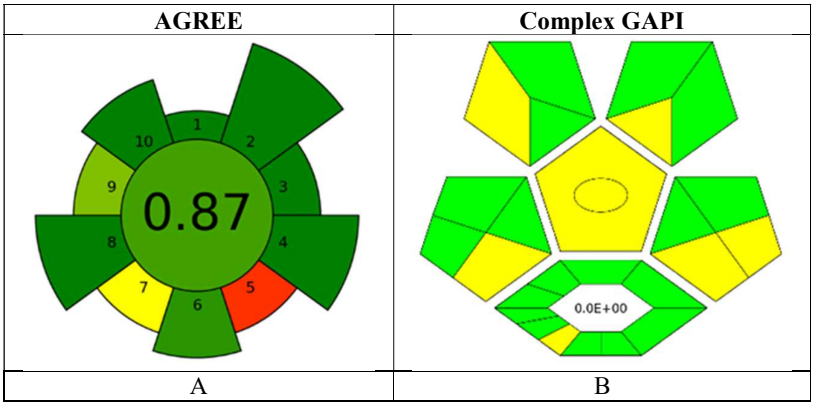


Figure 9: Greenness assessment of the analytical method using (A) AGREE metric and (B) Green Analytical Procedure Index (GAPI) pictogram

CONCLUSION

Utilizing PEG-400 plasticizer, the current study successfully designed and optimized SIT-loaded bioadhesive ocular films (SITOFs) utilizing a Box–Behnken Design-based QbD technique. The improved formulation SITOF-1 showed notable antibacterial activity, concentration-dependent antioxidant activity, controlled sustained drug release based on Higuchi diffusion kinetics, efficient ex vivo corneal penetration, and good physicochemical properties. In vivo studies confirmed the non-irritant profile and comparable anti-inflammatory efficacy of SITOF-1 relative to a corticosteroid standard, with 86.9% inflammation reduction at 72 h. Accelerated stability testing confirmed formulation robustness over 6 months. These results for ocular drug administration and show the viability and prospects for repurposing SIT as a topically delivered ophthalmic anti-inflammatory medication. To completely confirm the therapeutic potential of this approach, more research using pharmacokinetic studies and clinical evaluation is necessary.

Ethical statement

All animal experimental procedures were conducted in accordance with the ARRIVE guidelines and approved by the Institutional Animal Ethics Committee of Centurion University of Technology and Management, Bhubaneswar, India (Approval No. CUTM/IAEC/45). The study adhered to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, India.

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Conflict of interest

The authors declare that there is no conflict of interest.

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REFERENCES

1. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. American Association of Pharmaceutical Scientists. 2010; 12(3): 348-360. https://doi.org/10.1208/s12248-010-9183-3.
2. Azari AA, Arabi A. Conjunctivitis: A Systematic Review. Journal of Ophthalmic and Vision Research. 2020; 15(3): 372-395. https://doi.org/10.18502/jovr.v15i3.7456.
3. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: An Overview. World Journal of Pharmacology. 2013; 2(2): 47-64. https://doi.org/10.5497/wjp.v2.i2.47
4. Morrison PW, Khutoryanskiy VV. Advances in ophthalmic drug delivery. Therapeutic Delivery. 2014; 5(12): 1297-1315. https://doi.org/10.4155/tde.14.75
5. Grassiri B, Zambito Y, Bernkop-Schnürch A. Strategies to prolong the residence time of drug delivery systems on ocular surface. Advances in Colloid and Interface Science. 2021; 288: 102342. https://doi.org/10.1016/j.cis.2020.102342
6. Wafa HG, Essa EA, El-Sisi AE, El Maghraby GM. Ocular films versus film-forming liquid systems for enhanced ocular drug delivery. Drug Delivery and Translational Research. 2021; 11(3): 1084-1095. https://doi.org/10.1007/s13346-020-00825-1

7. Hermans K, Van den Plas D, Kerimova S, Carleer R, Adriaensens P, Weyenberg W, et al. Development and characterization of mucoadhesive chitosan films for ophthalmic delivery of cyclosporine A. International Journal of Pharmaceutics. 2014; 472(1-2): 10-19. https://doi.org/10.1016/j.ijpharm.2014.06.017

8. Tighsazzadeh M, Mitchell JC, Boateng JS. Development and evaluation of performance characteristics of timolol-loaded composite ocular films as potential delivery platforms for treatment of glaucoma. International Journal of Pharmaceutics. 2019; 566: 111-125. https://doi.org/10.1016/j.ijpharm.2019.05.059

9. Ayaloglu-Butun F, Peltek OO, Erdem Aslan A, Ozdemir T, Ayan S, Tiftikci U, et al. Anti-inflammatory effect of sitagliptin on lipopolysaccharide-induced activation of macrophages. Journal of Pharmacy and Pharmacology. 2021; 73(2): 196-204. https://doi.org/10.1093/jpp/rgaa009

10. Drucker DJ. The biology of incretin hormones. Cell Metabolism. 2006; 3(3): 153-165. https://doi.org/10.1016/j.cmet.2006.01.004

11. ICH Q8(R2). Pharmaceutical Development. International Council for Harmonisation; 2009. Assed on 26th Jun 2026.

12. Kumar LS, Ahad HA. Quality by Design-based quercetin hydrate nanoemulsions for enhanced solubility. Indian Journal of Pharmaceutical Education and Research. 2023; 57(4): 965-970. https://doi.org/10.5530/ijper.57.4.118

13. Kalam MA, Sultana Y, Ali A, Aqil M, Mishra AK, Aljuffali IA. Development and optimization of solid lipid nanoparticles using Box–Behnken statistical design for ocular delivery of Gatifloxacin. Journal of Biomedical Materials Research. Part A. 2013; 101(6), 1813-1827. https://doi.org/10.1002/jbm.a.34453

14. Al-Shaalan NH, Alam J, Alotaibi M, Alarifi A, Baazeem A. Sitagliptin: synthesis, spectroscopic characterization, and in vitro anti-inflammatory activity. Molecules. 2018; 23(3): 662. https://doi.org/10.3390/molecules23030662

15. Nandi S, Ojha A, Nanda A, Sahoo RN, Swain R, Pattnaik KP, et al. Vildagliptin plasticized hydrogel film in the control of ocular inflammation after topical application: study of hydration and erosion behaviour. Zeitschrift für Physikalische Chemie. 2022; 236(2): 275-290. https://doi.org/10.1515/zpch-2021-3081

16. Mahajan HS, Deshmukh SR. Development and evaluation of gel-forming ocular films based on Xyloglucan. Carbohydrate Polymers. 2015; 122: 243-247. https://doi.org/10.1016/j.carbpol.2015.01.020

17. Li B, Wang J, Gui Q, Yang H. Drug-loaded chitosan film prepared via facile solution casting and air-drying of plain water-based chitosan solution for ocular drug delivery. Bioactive Materials. 2020; 5(3): 577-583. https://doi.org/10.1016/j.bioactmat.2020.04.013

18. Mishra K, Dash A, Jabeen A, Vegesna S, Sahoo SK, Gupta V, Jena D. Chemometric Assisted UV-Spectrophotometric Quantification of Tigecycline in Parenteral Dosage Form. International Journal of Drug Delivery Technology. 2023;13(3):976-981.

19. Shastri D, Shelat P, Shukla A, Patel P. Ophthalmic drug delivery system: challenges and approaches. Systematic Reviews in Pharmacy. 2010; 1(2): 113-119. https://doi.org/10.4103/0975-8453.75042

20. Kumar A, Tiwari BK, Kumar S. Evaluation of ocular films of ofloxacin for antibacterial activity. International Journal of Applied Pharmaceutics. 2018; 10(6): 275-276. https://doi.org/10.22159/ijap.2018v10i6.27188

21. Yaribeygi H, Maleki M, Sathyapalan T, Sahebkar A. Anti-inflammatory potentials of incretin-based therapies used in the management of diabetes. Life Sciences. 2019; 241: 117152. https://doi.org/10.1016/j.lfs.2019.117152