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Title: Plackett-Burman design for screening of critical variables and their effects on the optical transparency and swelling of gatifloxacin-Pluronic-loaded contact lens

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Abstract: The optical and swelling properties of gatifloxacin-loaded contact lens decrease owing to the precipitation of gatifloxacin (on hydration) in the matrix structure of the contact lens. This paper focuses on the use of Pluronic F68 both inside and outside (in the packaging solution) the contact lens to form micelles to dissolve the gatifloxacin precipitates and not limited to sustain the release of gatifloxacin. The aim of this study was to screen the critical variables affecting the optical and swelling properties of gatifloxacin-loaded contact lens. The independent variables investigated were the concentration of Pluronic F68 incorporated in the monomer solution to fabricate the lens (X1, %w/v), the concentration of Pluronic F68 in the packaging solution (X2, %w/v), the concentration of gatifloxacin incorporated in the monomer solution (X3, %w/v), the concentration of gatifloxacin incorporated in the packaging solution during autoclave (X4, %w/v), the concentration of gatifloxacin incorporated in the packaging solution during extraction (X5, %w/v), the time (stabilization time) after the addition of gatifloxacin and Pluronic F68 to the monomer solution before the fabrication of the lens (X6, h), the pH of the packaging solution (X7), the temperature of the extracted solution (X8, °C), and the curing time for fabricating the contact lens (X9, min). The gatifloxacin-loaded contact lenses were characterized for their optical transmittances after sterilization on day 1 (Y1, %), optical transmittances after 7 days of sterilization (Y2, %) and swelling percentages after 7 days of sterilization (Y3, %). The selected variables showed responses that were in the ranges 53.5 % to 97.2 %, 51.3 % to 92.6 %, and 50.3 % to 83.7 % for Y1, Y2, and Y3, respectively. The data suggest that the presence of Pluronic F68 inside the contact lens (X1) reduced the optical and swelling properties of the contact lens, whereas the presence of Pluronic F68 in the packaging solution (X2) improved them

through micelle formation. The other variables (X3 to X9) did not exhibit significant effects on the swelling and transmittance. This study revealed the potential of Plackett-Burman design to screen the selected critical variables that affected the optical and swelling properties of gatifloxacin-loaded contact lens.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:

Data will be made available on request

Graphical Abstract

GATIFLOXACIN-LOADED CONTACT LENS

Reduction in Optical Transmittance and Swelling Property

% OPTICAL TRANSMITTANCE AFTER STERILIZATION ON DAY 1 (Y₁)

% SWELLING AFTER 7 DAYS OF STERILIZATION (Y₃)

GATIFLOXACIN-PLURONIC LOADED CONTACT LENS

Improvement in Optical Transmittance and Swelling Property [batch 4]

Plackett-Burman design for screening of critical variables and their effects on the optical transparency and swelling of gatifloxacin-Pluronic-loaded contact lens

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Abstract

The optical and swelling properties of gatifloxacin-loaded contact lens decrease owing to the precipitation of gatifloxacin (on hydration) in the matrix structure of the contact lens. This paper focuses on the use of Pluronic F68 both inside and outside (in the packaging solution) the contact lens to form micelles to dissolve the gatifloxacin precipitates and not limited to sustain the release of gatifloxacin. The aim of this study was to screen the critical variables affecting the optical and swelling properties of gatifloxacin-loaded contact lens. The independent variables investigated were the concentration of Pluronic F68 incorporated in the monomer solution to fabricate the lens (X_1 , %w/v), the concentration of Pluronic F68 in the packaging solution (X_2 , %w/v), the concentration of gatifloxacin incorporated in the monomer solution (X_3 , %w/v), the concentration of gatifloxacin incorporated in the packaging solution during autoclave (X_4 , %w/v), the concentration of gatifloxacin incorporated in the packaging solution during extraction (X_5 , %w/v), the time (stabilization time) after the addition of gatifloxacin and Pluronic F68 to the monomer solution before the fabrication of the lens (X_6 , h), the pH of the packaging solution (X_7), the temperature of the extracted solution (X_8 , °C), and the curing time for fabricating the contact lens (X_9 , min). The gatifloxacin-loaded contact lenses were characterized for their optical transmittances after sterilization on day 1 (Y_1 , %), optical transmittances after 7 days of sterilization (Y_2 , %) and swelling percentages after 7 days of sterilization (Y_3 , %). The selected variables showed responses that were in the ranges 53.5 % to 97.2 %, 51.3 % to 92.6 %, and 50.3 % to 83.7 % for Y_1 , Y_2 , and Y_3 , respectively. The data suggest that the presence of Pluronic F68 inside the contact lens (X_1) reduced the optical and swelling properties of the contact lens, whereas the presence of Pluronic F68 in the packaging solution (X_2) improved them through micelle formation. The other variables (X_3 to X_9) did not exhibit significant effects on the swelling and transmittance. This study revealed the potential of Plackett-Burman design to screen the selected critical variables that affected the optical and swelling properties of gatifloxacin-loaded contact lens.

Key words: Contact lenses, gatifloxacin, Pluronic F68, optical transmittance, swelling.

1. Introduction

The current market for ophthalmic drugs is dominated by eye drop solutions, ointment tubes, and in-situ gels, which address about 90 % of ocular diseases such as glaucoma, dry eye syndrome, and bacterial infections [1-6]. However, there are many known issues associated with these conventional dosage forms that, along with the anatomical and physiological barriers of the eye, result in poor ocular bioavailability (1-5 %) and patient non-compliance [1, 7, 8]. Therefore, a novel approach is needed to treat the eye diseases associated with the anterior segment that replaces the conventional dosage forms. Drug-loaded contact lenses can be used as a potential medical device to deliver drugs to the anterior segment of the eye [8, 9]. In the last few decades, drug-loaded contact lenses have received a great deal of attention owing to their biocompatibility, versatility, and low cost [10-14]. In comparison to multiple eye drop therapy, the ocular bioavailability can be increased many folds by using drug-loaded contact lenses [15, 16]. However, the incorporation of the drug-like gatifloxacin in the contact lens reduces the optical transmittance and swelling properties owing to the precipitation of the drug in the matrix of the contact lens.

Patrick et al., 2009 fabricated a contact lens by using a poly(ethylene glycol)-based copolymer containing multiple thiol groups for controlled ocular delivery of pilocarpine. An increase in the copolymer concentration reduced the transparency and swelling of the contact lens. The system showed biphasic release profile over 8 days, with an eight-fold increase in the duration of action in a rabbit model study [17]. Chauhan et al., 2006 observed a drastic reduction in the transparency and swelling properties of hydrogels when lidocaine and polymerizing mixtures were incorporated at different ratios [18]. Gulsen et al., 2005 stabilized drug microemulsions in poly-2-hydroxyethyl methacrylate hydrogels, however, a loss in the optical transparency was observed, due to the aggregation of the oil globules [19]. In another study, Gulsen et al., 2004 incorporated lidocaine microemulsion in contact lenses, which resulted in a reduction in the optical transmittance of up to 66 %w/w [20]. Kim et al., 2008 developed an ophthalmic drug loaded extended wear soft contact lens. The results showed reductions in the optical properties with increases in the N,N-dimethylacrylamide levels [21]. Paradiso et al., 2016 developed levofloxacin-loaded ACUVUE® contact lenses by including vitamin E as

a barrier. An increase in the weight of the vitamin up to 20 %w/w caused a significant reduction in the transmittance of the contact lens [22]. To treat allergic diseases, Gonzalez et al., 2016 developed a biomimetic contact lens by using 2-acrylamido-2-methyl-1-propane-sulfonic acid and benzyl methacrylate as the functional monomers [23]. Copolymerization with benzyl methacrylate led to a marked decrease in the swelling behavior of the lens. Malakooti et al., 2015 applied molecular imprinting to load polymyxin B and vancomycin into contact lens. The lens appeared translucent (45 % to 15 %w/w) with an increase in the level of polymyxin B [24]. Shayani et al., 2017 developed extended wear contact lenses loaded with ciprofloxacin and betamethasone and observed a significant reduction in the water content of the contact lenses due to the presence of vitamin E [25].

In spite of the successful demonstration of therapeutic contact lenses, the incorporation of drugs (like gatifloxacin) in the contact lens reduces the optical transmittance and swelling properties owing to the precipitation of the drug in the matrix of the contact lens. The preliminary data (shown in supplementary material A) indicate that the optical transmittance and swelling of gatifloxacin-loaded contact lens are 83.5 ± 7.2 % and 63.3 ± 3.1 %, respectively. The focus of this study was to improve the critical lens properties by using Pluronic F68, to produce micelles for dissolving the gatifloxacin precipitates present in the matrix of the contact lens material. The presence of Pluronic F68 both inside and outside (in the packaging solution) the contact lens can generate micelles that dissolve the gatifloxacin precipitates and can also promote sustained release of the drug from the contact lens. This study hypothesized that the improvement in the optical and swelling properties of gatifloxacin-loaded contact lenses is due to the presence of the Pluronic F68 micelles. Plackett-Burman (PB) design was applied to screen the critical variables affecting the optical transparency and swelling of the gatifloxacin-loaded contact lenses.

2. Materials and methods

2.1. Materials

Pluronic F68, hydroxyl ethyl methacrylate (HEMA), Irgacure[®] (1-hydroxycyclohexyl phenyl ketone), ethylene glycol dimethacrylate (EGDMA), N-vinyl pyrrolidone (NVP),

N,N-dimethyl acrylamide (DMA), and 3-[tris(trimethylsiloxy)silyl] propyl methacrylate (siloxane) were all supplied by Sigma-Aldrich Chemicals (St. Louis, MO, USA). Gatifloxacin was gifted by Sun Pharma Ind. Ltd. (Gujarat, India).

2.2. Design of experiments

The PB design was used to investigate large numbers of variables simultaneously in a few experimental runs and identify the most critical variables with high predictabilities [26-29]. The design was two-level (-1 and +1) and employed to screen “n” variables in “n+1” experiments. The independent variables selected for the present investigation and their decoded values (-1 and + 1) are summarized in Table 1. The nine independent factors (plus two dummy) and their levels were selected based on preliminary experiments. The variables (12 experiments) in the PB design (Table 2) were investigated at two levels by using JMP trial version 7.0.1 (SAS, NC, USA) [30]. The linear equation of the PB model is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 \dots \dots \dots + b_nX_n \text{ ---- (1)}$$

where Y is the response, b_0 is a constant, and $b_1, b_2 \dots b_n$ are the partial regression coefficients of factors $X_1, X_2 \dots X_n$ (that represent the effect of each factor ordered within -1, +1). The dependent variables (responses) are the optical transmittance after sterilization on day 1 (Y_1 , %), the optical transmittance after 7 days of sterilization (Y_2 , %), and the % swelling after 7 days of sterilization (Y_3 , %).

Table 1
Experimental factors and their levels

Factor	Significance of the factor	Level (-1)	Level (+1)
X_1	Concentration of Pluronic F68 incorporated in the monomer solution for fabricating the lens (%w/v)	0.1	1
X_2	Concentration of Pluronic F68 in the packaging solution (%w/v)	0.5	1
X_3	Concentration of gatifloxacin incorporated in the monomer solution for fabricating the lens (%w/v)	0.3	0.5
X_4	Concentration of gatifloxacin in the packaging solution during autoclave (%w/v)	0.1	0.3
X_5	Concentration of gatifloxacin in the solution during extraction (%w/v)	0.1	0.3
X_6	Time (stabilization time) after the addition of gatifloxacin and Pluronic F68 to the monomer solution before fabrication of the lens (h)	1	24
X_7	pH of the packaging solution	7.4	7.8
X_8	Temperature of the extraction solution (°C)	50	100
X_9	Curing time to fabricate the lens (min)	45	65

Table 2

PB design

Batch	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉
1	-1	1	1	1	-1	-1	1	-1	1
2	1	1	1	-1	-1	1	-1	1	1
3	1	1	-1	1	1	-1	-1	-1	1
4	-1	-1	-1	1	-1	1	-1	1	1
5	-1	1	-1	1	1	1	1	1	-1
6	1	1	-1	-1	-1	-1	1	1	-1
7	1	-1	1	1	1	-1	-1	1	-1
8	1	-1	-1	-1	1	1	1	-1	1
9	-1	1	1	-1	1	1	-1	-1	-1
10	-1	-1	1	-1	1	-1	1	1	1
11	1	-1	1	1	-1	1	1	-1	-1
12	-1	-1	-1	-1	-1	-1	-1	-1	-1

X₁ = Concentration of Pluronic F68 incorporated in the monomer solution for fabricating the lens (%w/v)

X₂ = Concentration of Pluronic F68 in the packaging solution (%w/v)

X₃ = Concentration of gatifloxacin incorporated in the monomer solution for fabricating the lens (%w/v)

X₄ = Concentration of gatifloxacin in the packaging solution during autoclave (%w/v)

X₅ = Concentration of gatifloxacin in the solution during extraction (%w/v)

X₆ = Time (stabilization time) after the addition of gatifloxacin and Pluronic F68 to the monomer solution before fabrication of the lens (h)

X₇ = pH of the packaging solution

X₈ = Temperature of the extraction solution (°C)

X₉ = Curing time to fabricate the lens (min)

The study was designed to investigate the effect of critical variables on the optical and swelling properties of gatifloxacin-loaded contact lens. The target was to minimize the change in the % swelling and achieve >95 % optical transmittance [31] of the gatifloxacin-loaded contact lens by using the selected monomer composition. The % swelling and optical transmittance of the control blank contact lens (without a drug) were 85.4 % and 99.7 %, respectively. The presence of Pluronic F68 inside (X₁) and outside (in the packaging solution, X₂) the contact lens can form micelles [30] that dissolve the gatifloxacin precipitates inside the lens matrix. The solubility of gatifloxacin in simulated tear fluid (STF) is 3.66 mg/ml, and an improvement in the solubility to 13.62 mg/ml was observed in the presence of Pluronic F68 (0.5 %w/v). The critical micelle concentration (CMC) of Pluronic F68 in the range 20 to 25 °C is 0.33 %w/v. Therefore, this study hypothesized that the improvements in the optical and swelling properties of gatifloxacin-loaded contact lenses are due to the presence of Pluronic F68 micelles. The concentration of gatifloxacin incorporated in the monomer solution for fabrication of the lens (X₃) was based on the drug loading required to treat bacterial conjunctivitis (70

to 120 µg for 3 days; assay values obtained from preliminary trails). However, the dose selection was based on the prediction, considering the sustained release of gatifloxacin from the contact lens [32]. The concentration of gatifloxacin incorporated in the packaging solution during the autoclave (X_4) and extraction (X_5) was restricted to 0.3 %w/v, considering the highest strength of gatifloxacin eye drops available in the market [33]. The presence of the drug in the extraction and autoclave solutions will promote its uptake by the contact lens [21]. X_6 was selected as the stabilization time (equilibrium time) required after the addition of gatifloxacin and Pluronic F68 to the monomer solution before the fabrication of the lens. The hypothesis was the stable Pluronic F68 micelles formed (in the monomer solution) over time encapsulated the gatifloxacin before the fabrication of the lens. Mostly, contact lenses are supplied in a packaging solution with pH 7.4 [34]. However, we can increase the pH up to 7.8 (without causing much eye irritation) to increase the swelling property of contact lens [35, 36] in order to compensate for the reduction in swelling due to the presence of gatifloxacin. In this study, we have selected two approaches to remove unreacted monomers from the contact lens matrix after fabrication that involve two different temperatures (50 and 100 °C) to investigate its effect on the optical and swelling properties of gatifloxacin-loaded contact lens [37, 38]. The curing (polymerization) time to fabricate the contact lens (X_9 , min) was in the range 45 to 65 min. A time below 45 min resulted in poor mechanical strength of the lens, whereas a duration above 65 min produced brittleness in the fabricated lens matrix [39]. The thickness of the contact lens was 100 µm.

2.3. Fabrication of the contact lenses

Twelve batches of the PB design were developed by free radical polymerization technique according to the compositions listed in Table 2 by using a monomer mixture [9, 40]. The composition of the monomer mixture was 62.50 %w/v HEMA, 31 %w/v DMA, 1 %w/v EGDMA, 1 %w/v NVP, 2.5 %w/v 3-[tris(trimethylsiloxy)silyl] propyl methacrylate (siloxane), and 2 %w/v photo-initiator (Irgacure). The contact lenses were fabricated by the cast molding technique using polypropylene molds. To fabricate the contact lens, the required concentrations of gatifloxacin and Pluronic F68 (table 2) were added to the monomer mixture. This mixture was left to attain equilibrium over two

different time intervals (stabilization times of 1 and 24 h) that provided sufficient time for the formation of stable micelles of Pluronic F68 inside the monomer solution for encapsulating the drug. After the equilibrium time, the excess monomer mixture solution was added to the female mold, and the male mold was placed, which was followed by curing in an ultraviolet transilluminator at 360-370 nm for 45 and 65 min, as per the screening design batches. The contact lenses were removed from the molds and stored in desiccators (45 % humidity) for further study.

2.4. Extraction of the contact lenses to remove the unreacted monomers

In this study, two approaches were used to remove the unreacted monomers from the contact lens matrix after the fabrication of the lens that involved two different temperatures (50 and 100 °C). This enabled us to investigate its effect on the optical and swelling properties of the gatifloxacin-loaded contact lens. The conditions were applied according to the design matrix. In the first approach, the contact lens was extracted (individually) by placing the individual lenses in water at 100 °C for 15 min [41, 42]. In the second approach, the extraction was performed at 50 °C in a buffer of pH 8 (5 g/l NaHCO₃ and 0.05 g/l Pluronic F127 in deionized water) for 30 min. According to Table 2, gatifloxacin (0.1 or 0.3 %w/v) was added to the extraction solution to maintain the equilibrium of the drug between the lens matrix and the extraction solution. This step was included to prevent drug leaching or additional uptake of the drug by the contact lens during the extraction.

2.5. Sterilization and packaging of the contact lenses

After the extraction step, the contact lenses were sterilized in the packaging solution by autoclave (at 121 °C and 15 psi for 15 min) [43]. The packaging solution was composed of 6.78 g/l NaCl, 2.18 g/l NaHCO₃, 1.38 g/l KCl, and 0.084 g/l CaCl₂·2H₂O in deionized water (also called as STF), along with gatifloxacin (0.1 or 0.3 %w/v), according to Table 2. Gatifloxacin was added to the packaging solution during the sterilization to maintain the equilibrium of gatifloxacin between the lens matrix and the packaging solution. This could prevent drug leaching or facilitate additional uptake of

gatifloxacin by the contact lens [21, 44, 45]. The pH of the packaging solution was adjusted at two levels (7.4 and 7.8).

2.6. Evaluation of the contact lenses

2.6.1. Swelling study

The contact lenses were removed from the mold and accurately weighed (dry weight = W_D), which was followed by the extraction and sterilization steps, as discussed in the above sections (as per the PB design). After 7 days, the contact lenses were removed from the packaging solution and weighed (W_S). The swelling study was carried out after 7 days to provide sufficient time for the Pluronic F68 micelles to encapsulate the drug and to ensure equilibrium of the drug between the packaging solution and the lens matrix. The % swelling [46, 47] was calculated by using the following formula:

$$\% \text{ Swelling} = \frac{W_S - W_D}{W_D} \times 100 \text{ ----- (2)}$$

2.6.2. Optical transmittance study

The optical transmittances of the contact lenses should not change [48-52] owing to the presence of gatifloxacin. The transmittance study was carried out by using a UV-vis spectrophotometer. The contact lenses were removed from the packaging solution (on day 1 and after 7 days of autoclave) and positioned inside the quartz cuvette, which was placed in the UV-vis spectrophotometer to measure the transmittance in the range 200-800 nm [53]. The % transmittance was also measured at 600 nm for investigation and comparison.

2.7. Statistical analysis

The PB design analysis was performed by using JMP trial version 7.0.1 (SAS, NC, USA).

3. Results and discussion

3.1. Influence of the investigated variables on the optical transmittance on day 1 after sterilization (Y_1)

On day 1, after sterilization, the optical transmittance varied from 53.5 ± 2.8 % (batch 7) to 97.2 ± 2.5 % (batch 10) (Table 3). The concentration of Pluronic F68 in the monomer solution (X_1 ; %w/v) showed the most significant negative effect ($b_1 = -12.18$, $p < 0.01$) on the optical transmittance (Table 4, Fig 1(A & B)). The negative value of the coefficient suggests a reduction in the optical transmittance with an increase in the concentration of Pluronic F68 in the monomer solution (X_1). The linear model explaining the influence of the variables on the optical transmittance (Y_1) is given as

$$Y_1 = 71.94 - 12.18X_1 - 1.40X_2 + 0.20X_3 - 5.06X_4 + 1.83X_5 - 1.54X_6 + 1.89X_7 - 1.56X_8 + 0.26X_9 \quad \text{--- (3)}$$

ANOVA results suggest a significant effect ($F = 35.92$, $p < 0.01$) of the selected independent variables on the optical transmittance on day 1 of sterilization (Y_1) (Table 5). The R^2 value is found to be 0.959, which suggests that 95.9 % variation in the response (Y_1) is observed because of the selected independent variables (X_1 to X_9).

Fig. 1. Contour plots illustrating the effects of changing independent variables on the optical transmittance and swelling properties of contact lenses.

Table 3

Observed and predicted values of % optical transmittance after sterilization on day 1 (Y_1), % optical transmittance after 7 days of sterilization (Y_2), and % swelling after 7 days of sterilization (Y_3).

Batch	Observed Value (Y_1)	Predicted value (Y_1)	Observed value (Y_2)	Predicted value (Y_2)	Observed value (Y_3)	Predicted value (Y_3)
1	78.8 ± 2.5	81.3	84.4 ± 8.6	87.9	69.9 ± 5.5	72.7
2	53.9 ± 7.1	57.1	67.9 ± 6.3	72.9	63.6 ± 3.2	67.9
3	58.9 ± 6.8	56.4	61.2 ± 5.6	57.6	78.5 ± 9.1	75.6
4	76.8 ± 2.6	73.7	91.4 ± 4.4	86.4	72.7 ± 7.5	68.4
5	74.7 ± 8.2	77.8	84.2 ± 2.5	89.2	82.7 ± 3.8	87.0
6	66.2 ± 5.6	63.0	76.2 ± 2.4	71.2	73.9 ± 3.0	69.6
7	53.5 ± 2.8	55.9	51.3 ± 3.4	54.9	50.5 ± 9.4	53.4
8	67.6 ± 5.7	70.1	63.2 ± 7.1	66.8	62.6 ± 4.9	65.4
9	90.8 ± 2.9	87.7	91.6 ± 7.9	86.6	83.7 ± 5.5	79.4
10	97.2 ± 2.5	94.7	92.6 ± 2.5	88.9	71.4 ± 5.5	68.5
11	58.6 ± 4.0	56.1	66.7 ± 2.1	63.1	50.3 ± 7.9	47.4
12	86.4 ± 5.1	89.5	77.1 ± 2.4	82.1	58.2 ± 6.3	62.5

Table 4

Results of statistical analyses of % optical transmittance after sterilization on day 1 (Y_1), % optical transmittance after 7 days of sterilization (Y_2), and % swelling after 7 days of sterilization (Y_3).

Factor	% T after sterilization (day 1)		% T after sterilization (day 7)		% swelling after equilibrium (day 7)	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Intercept	71.94	<0.01	75.65	<0.01	68.15	<0.01
X_1	-12.18	<0.01	-11.23	0.01	-4.93	0.12
X_2	-1.40	0.62	1.94	0.46	7.23	0.04
X_3	0.20	0.94	0.10	0.97	-3.26	0.26
X_4	-5.06	0.06	-2.44	0.35	-0.74	0.83
X_5	1.83	0.43	-1.63	0.61	3.41	0.24
X_6	-1.54	0.58	1.86	0.56	1.09	0.75
X_7	1.89	0.41	2.23	0.39	0.29	0.94
X_8	-1.56	0.58	1.63	0.61	0.99	0.77
X_9	0.26	0.93	1.14	0.73	1.60	0.64

Table 5

ANOVA results of % optical transmittance after sterilization on day 1 (Y_1), % optical transmittance after 7 days of sterilization (Y_2), and % swelling after 7 days of sterilization (Y_3).

Response	Source	DF	Sum of squares	Mean of squares	F ratio	Prob > F*
Y_1	Model	2	2086.50	1043.25	35.92	< 0.01
	Error	9	261.38	29.04		
	Cumulative total	11	2347.88			
Y_2	Model	1	1515.38	1515.38	28.77	< 0.01
	Error	10	526.66	52.67		
	Cumulative total	11	2042.04			
Y_3	Model	1	627.42	627.42	8.02	0.01
	Error	10	782.74	78.27		
	Cumulative total	11	1410.16			

*Prob > F is the significance level, and a value less than 0.05 is considered as significant.

The optical transmittance and swelling of the gatifloxacin-loaded contact lens (0.5 %w/v gatifloxacin in the monomer solution and 0.3 %w/v gatifloxacin in the packaging solution) were 83.5 ± 7.2 % and 63.3 ± 3.1 %, respectively. The present study aimed to improve the critical lens properties by using Pluronic F68 to form micelles to dissolve the gatifloxacin precipitates inside the lens matrix and to promote sustained drug release from the contact lens. This research revealed a reduction in the optical transmittance if Pluronic F68 was present inside the contact lens (X_1). The unexpected reduction was attributed to the drug-Pluronic-monomer interaction occurring during the curing process. The presence of Pluronic F68 outside the contact lens, i.e., in the packaging solution (X_2) did not result in a significant effect ($b_2 = -1.40$, $p = 0.62$) in terms of improving the optical transmittance after sterilization on day 1. The increase in the concentration of gatifloxacin in the packaging solution during autoclave (X_4 ; $b_4 = -5.06$, $p = 0.06$) reduced the optical transmittance, which was expected and due to the high uptake of the hydrophobic drug by the contact lens matrix structure. The variable X_3 (concentration of gatifloxacin in the monomer solution) did not exhibit a significant effect ($b_3 = 0.20$, $p = 0.94$) on the optical transmittance, however, the optical transmittance was below the requirement (of >95 %). The other variables like the concentration of gatifloxacin incorporated in the solution during the extraction (X_5), the time (stabilization time) after the addition of gatifloxacin and Pluronic F68 to the monomer solution before

the fabrication of the lens (X_6), the pH of the packaging solution (X_7), the temperature of the extraction solution (X_8), and the curing time to fabricate the lens did not show significant effects on the optical transmittance.

3.2. Influence of the investigated variables on the optical transmittance after 7 days of sterilization (Y_2)

The optical transmittances of the contact lenses were also measured after 7 days of sterilization, based on the hypothesis that, with time, Pluronic F68 will form stable micelles that dissolve the precipitates of gatifloxacin in the contact lens matrix, which will lead to improvements in the optical and swelling properties of the lens. The optical transmittances of the contact lenses after 7 days of sterilization varied from 51.3 ± 3.4 % (batch 7) to 92.6 ± 2.5 % (batch 10) for the nine selected independent variables (Table 3). The linear model explaining the influence of the variables on the optical transmittance (Y_2) is given by

$$Y_2 = 75.65 - 11.23X_1 + 1.94X_2 + 0.10X_3 - 2.44X_4 - 1.63X_5 + 1.86X_6 + 2.23X_7 + 1.63X_8 + 1.14X_9 \quad (4)$$

The ANOVA results suggest the significant effect ($F = 28.77$, $p < 0.01$) of the selected independent variables on the optical transmittance after 7 days of sterilization (Y_2) (Table 5). The R^2 value is found to be 0.887, which suggests that 88.7 % variation in the response (Y_2) is observed because of the selected independent variables (X_1 to X_9). The optical transmittance due to the presence of Pluronic F68 inside the contact lens (X_1) still showed a significant negative effect ($b_1 = -11.23$, $p = 0.01$) on the 7th day of sterilization (Table 4, Fig 1(C & D)). Interestingly, after 7 days of sterilization, the presence of Pluronic F68 in the packaging solution (X_2) resulted in a 3.34 magnitude improvement ($b_2 = +1.94$, positive effect) in the optical transmittance of the contact lens from days 1 to 7. The results support the hypothesis that the presence of Pluronic F68 in the packaging solution aids the formation of micelles, which migrate inside the contact lens matrix to dissolve the gatifloxacin precipitates and improve the optical transmittance of the contact lens. This hypothesis was further supported through determination of the size of the micelles extracted from the contact lens. For more details, please refer to supplementary material B. The other variables (X_3 to X_9) did not display significant effects on the optical transmittance after 7 days of sterilization.

3.3 Influence of the investigated variables on the % swelling after 7 days of sterilization (Y_3)

The swellings of the contact lenses were measured after 7 days of sterilization, based on the hypothesis that Pluronic F68 will form stable micelles that encapsulate the gatifloxacin to improve the swelling properties of the contact lens. The % swellings of the contact lenses varied from 50.3 ± 7.9 % (batch 11) to 83.7 ± 5.5 % (batch 9) (Table 3). The linear model explaining the influence of various variables on % swelling (Y_3) is given by

$$Y_3 = 68.15 - 4.93X_1 + 7.23X_2 - 3.26X_3 - 0.74X_4 + 3.41X_5 + 1.09X_6 + 0.29X_7 + 0.99X_8 + 1.60X_9 \quad \text{--- (5)}$$

The ANOVA results suggest a significant effect ($F = 8.02$, $p < 0.01$) of the selected independent variables on the % swelling after 7 days of sterilization (Y_3) (Table 5). The R^2 value is found to be 0.886, which suggests that 88.6 % variation in the response (Y_3) is observed because of the selected independent variables (X_1 to X_9). The presence of Pluronic F68 in the packaging solution (X_2) results in a significant improvement in the % swelling of the contact lens ($b_2 = +7.23$, $p = 0.04$, positive impact). The positive sign of the coefficient suggests that a unit increment in the concentration of Pluronic F68 in the packaging solution results in an increase of 7.23 units in the % swelling. The presence of Pluronic F68 inside the contact lens (X_1) has a negative effect ($b_1 = -4.93$) on the % swelling. However, it is not significant ($p = 0.12$) (Table 4, Fig 1 (E and F)). Similarly, the gatifloxacin concentrations in the monomer (X_3) and packaging solution (X_4) show negative effects on the swelling property of the contact lens, which is expected. The remaining variables (X_5 to X_9) do not exhibit significant effects on the % swelling.

Additionally, thermal degradation, drug integrity, and drug release profile studies were conducted with batch 9, which showed the best results (transmittance of 91.6 % and swelling of 83.7 %). The thermal degradation study revealed an insignificant difference ($p > 0.01$) before and after the sterilization of the packaging solution (STF + gatifloxacin + Pluronic F68, batch 9), which suggested that the gatifloxacin was thermally stable during the autoclave. Microbiological assay of the drug eluted from the contact lens confirmed the chemical stability of the gatifloxacin (for more details, please see supplementary material C). The total drug uptake (drug loading) and drug release profile data suggest improvements in the release profiles of the gatifloxacin-Pluronic-

loaded contact lenses, in comparison to those of gatifloxacin-loaded contact lenses without Pluronic F68. For more details, please see supplementary material D.

4. Conclusion

Gatifloxacin-loaded contact lens displayed reduced optical ($83.5 \pm 7.2 \%$) and swelling ($63.3 \pm 3.1 \%$) properties due to the precipitation of gatifloxacin in the contact lens matrix. Therefore, Pluronic F68 was used to improve the critical lens properties of gatifloxacin-loaded contact lens. PB design was applied to screen the critical variables and study their effects on the optical transmittance and swelling of gatifloxacin-Pluronic-loaded contact lens. The study revealed that the presence of Pluronic F68 inside the contact lens (X_1) reduced the optical and swelling properties of the lens, therefore, one should avoid the use of Pluronic F68 in the monomers. The presence of gatifloxacin in the packaging solution during autoclave (X_4) also reduced the optical and swelling properties of the contact lens through drug uptake. On the other hand, the presence of Pluronic F68 in the packaging solution (X_2) improved the optical transmittance and % swelling after 7 days of sterilization. The data supports the hypothesis that Pluronic F68 forms micelles over time that dissolve the gatifloxacin precipitates in the contact lens matrix. The other variables (X_3 to X_9) displayed no significant effects on the optical transmittance and swelling properties. Further studies are in progress to optimize (3^2 factorial design) the levels of X_2 and X_4 to achieve the required values of % optical transmittance ($>95 \%$) and % swelling ($>95 \%$). The presence of the drug in the packaging solution during sterilization is mandatory to compensate for drug leaching from the gatifloxacin-Pluronic-loaded contact lens during autoclave and storage. In conclusion, the study revealed the potential of the PB design to screen and understand the effects of the selected variables on the critical lens properties.

Disclosures

There are no potential conflicts of interest to disclose for this work.

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