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Research article

In situ ophthalmic gel forming systems of poloxamer 407 and hydroxypropyl methyl cellulose mixtures for sustained ocular delivery of chloramphenicole: optimization study by factorial design

Insan Sunan Kurniawansyah [a,*], Taofik Rusdiana [a], Iyan Sopyan [a], Handrian Ramoko [a], Habibah A. Wahab [b], Anas Subarnas [c,**]

[a] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, West Java, Indonesia
[b] School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia
[c] Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, West Java, Indonesia

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ABSTRACT

Background: Conventional drug delivery systems have some major drawbacks such as low bioavailability, short residence time and rapid precorneal drainage. An *in situ* gel drug delivery system provides several benefits, such as prolonged pharmacological duration of action, simpler production techniques, and low cost of manufacturing. This research aims to get the optimum formula of chloramphenicol *in situ* gel based on the physical evaluation.
Methods: The effects of independent variables (poloxamer 407 and hydroxypropyl methyl cellulose (HPMC) concentration) on various dependent variables (gelling capacity, pH and viscosity) were investigated by using 3^2 factorial design and organoleptic evaluation was done with descriptive analysis.
Results: The optimized formula of chloramphenicol *in situ* gel yielded 9 variations of poloxamer 407 and HPMC bases composition in % w/v as follows, F1 (5; 0.45), F2 (7.5; 0.45), F3 (10; 0.45), F4 (5; 0.725), F5 (7.5; 0.725), F6 (10; 0.725), F7 (5; 1), F8 (7.5; 1), F9 (10; 1). The results indicated that the organoleptic, pH, and gelling capacity parameters matched all formulas (F1–F9), however, the viscosity parameter only matched F3, F6, F8, and F9. Based on factorial design, F6 had the best formula with desirability value of 0.54, but the design recommended that formula with the composition bases of poloxamer 407 and HPMC at the ratio of 8.16 % w/v and 0.77 % w/v, respectively, was the optimum formula with a desirability value of 0.69.
Conclusion: All formulas have met the Indonesian pharmacopoeia requirements based on the physical evaluation, especially formula 6 (F6), which was supported by the result of factorial design analysis.

1. Introduction

Ophthalmic drug delivery systems constitute challenging research nowadays because of the eye's unique anatomy. Many eye disorders are treated by the use of topical medications in the form of eye drops. This preparation is often used because the price is low, and the usage is easy. However, these preparations have some major drawbacks, such as short residence time, low bioavailability, and rapid precorneal drainage. The low bioavailability of drugs from the conventional delivery system (i.e., eye drops) is due to a large extent to nasolacrimal drainage precorneal drug loss. The rapid clearance into the eye of the topically applied drug often results in poor therapeutic response, hence the need for a frequent dosing regimen [1, 2, 3, 4, 5]. A high-frequency eye drop dosing regimen is associated with patient non-adherence [6]. Due to these drawbacks, long-acting ophthalmic drug delivery systems are required for better patient adherence, improved local bioavailability, and reduced dose concentration and dosing frequency of administration [7, 8]. To overcome the lack of eye drops, an alternative type of ophthalmic *in situ* gel has been developed.

Ophthalmic *in situ* gel is able to change into gel when applied to the eye. An *in situ* gel drug delivery system provides several benefits, such as prolonged pharmacological duration of action, simpler production techniques, and low cost of manufacturing as compared to conventional drug delivery systems [9, 10]. The *in situ* gelling system consists of a

* Corresponding author.
** Corresponding author.
E-mail addresses: insan.sunan.kurniawansyah@unpad.ac.id (I.S. Kurniawansyah), aasubarnas@yahoo.co.id (A. Subarnas).

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stimuli-responsive polymer which displays sol-to-gel phase transitions in the eye due to a change in specific physicochemical parameters like pH, temperature, and electrolyte composition in the eye environment [11, 12, 13, 14, 15].

Poloxamer 407 is a well-known stimuli-responsive polymer type with thermoresponsive behaviour. It is commonly used as an eye drug delivery system as it could prolong drug release for eye tissue [16]. However, the major drawback of poloxamer 407 alone is low mucoadhesive activity [17]. In addition, adding excessive concentrations of poloxamer 407 can induce hypertriglyceridemia in the eye [18]. The addition of hydroxypropyl methyl cellulose (HPMC) can reduce the concentration of poloxamer 407 needed to form *in situ* gel gelation [16, 19]. Thereby, improving mucoadhesive activity and reducing the risk of hypertriglyceridemia induction in the eye.

Optimization of formulations using design of experiments (DOE) is a powerful and efficient tool in the development of pharmaceutical dosage forms. Quality by Design (QbD) uses multivariate analysis to understand the relationship between processing parameters (factors) and the selected responses through DOE with the smallest number of experiments, thereby reducing the time required for developing pharmaceutical formulation [20, 21, 22]. Based on the DOE, response surface methodology (RSM) has been used for optimization of various pharmaceutical formulation. RSM generates the polynomial equations of the response to determine the optimum formulation, and it allows evaluating the effects of multiple factors and their interactions on one or more response variables it is a useful method [22]. RSM can provide the best poloxamer 407 and HPMC composition solutions based on the physical parameters (F_{pred}). After determining the pH and viscosity requirements on the ophthalmic *in situ* gel, this software can provide a solution based on desirability value. Data analysis was performed based on optimization results with a three-level factorial design of the response surface method, because this design is allows us to study the effect of a single factor or combination (Poloxamer 407 and HPMC) to be estimated at several levels relative to the other factors (organoleptic, gelling capacity, pH and viscosity) [23]. Factorial designs are used primarily for understanding if factors are important to the process. This can take the form of screening for few important factors out of many possibilities, or characterizing how known factors interact and individually effect the process. These designs are often used as a starting point for more complex response surface modeling [24].

The aim of this research was to find the best chloramphenicol *in situ* gel formula composition based on its physical parameters by optimizing the combination basis of poloxamer 407 and HPMC. Before formulation, it was necessary to optimize the ophthalmic chloramphenicol *in situ* gel formulation. Optimization was done by using three-level factorial experimental design with response surface methodology. The optimized formula were evaluated based on their physical parameters (organoleptic, pH, viscosity, and gelling capacity) [24, 25], and all parameters were evaluated for formula optimization, but only pH and viscosity that were evaluated for data analysis with factorial design.

2. Materials and methods

2.1. Chemicals

The chemicals used were chloramphenicol antibiotic (Bio Basic Inc., Markham Ontario, Canada), hydroxypropyl methyl cellulose (HPMC) (KERRY, Zhoucun Plant, Shandong, China), calcium chloride dihydrate (Merck, Indonesia), sodium chloride (NaCl) (Merck, Indonesia), sodium bicarbonate (Merck, Indonesia), benzalkonium chloride (Merck, Indonesia), poloxamer 407 (Kolliphor® P 407, BASF Indonesia), aqua pro injection (Ikapharmindo Putramas, Indonesia), 70% ethanol (Ikapharmindo Putramas, Indonesia) and propylenglycol (Ikapharmindo Putramas, Indonesia).

2.2. Optimization of ophthalmic chloramphenicol in situ gel formula

The optimization was performed on the poloxamer 407 and HPMC bases by using a 3^2 factorial design. The concentration of poloxamer 407 (X_1) and concentration of HPMC (X_2) were chosen as independent variables in 3^2 full factorial designs, while pH (Y_1) and viscosity (Y_2) were chosen as dependent variables. The real values at the lower, middle, and upper levels of X_1 were 5%, 7.5%, and 10% and X_2 were 0.45%, 0.725% and 1%.

2.3. Formulation of ophthalmic chloramphenicol in situ gel

The optimization of formula was performed on poloxamer 407 and HPMC bases by using a 3^2 factorial design. This experimental design is able to study the effect of a single factor and interactions between factors on the values of responses (dependent variables). This design could provide a more accurate regression equation than the 2^k model due to more data presentation. Optimization with a three-level factorial resulted in 9 variations of the formulas, as listed in Table 1.

Table 1 showed the optimized formula for poloxamer 407 dan HPMC that had been added to active substance and excipients whose functions are known respectively. After that, all formulas were made according to the procedure. After the preparations were made, physical evaluations were carried out.

2.4. Procedure for chloramphenicol in situ gel formulation

The formulation process has been carried out aseptically in a laminar air flow (LAF) room that was sterilized with 70% alcohol. After cleaning, the UV lamp was turned on for 1.5 h. After that, the neon light and the blower were turned on.

Each formula was made aseptically. The poloxamer 407 and HPMC bases were made by dissolving each substance in hot aquadest in a separate container. The poloxamer 407 was dissolved continuously and gradually with slow stirring to prevent the formation of foam. It was dissolved in aqueous phase that has been heated to 70–90 °C. The HPMC base was made by putting the required amount of hot water (approximately 70 °C) into the container. HPMC was added gradually and we waited for it to float on the surface of water. After each polymer was dissolved, and the two polymers were mixed and stirred until homogeneous. The mixed base was cooled to support the gelling process. Then the mixed base was sterilized with an autoclave at 121 °C for 15 min [25]. The autoclave sterilized base was mixed with a mixture of chloramphenicol, propylenglycol and benzalkonium chloride that had been sterilized before with bacterial filter (0.22 μm) until homogeneous.

2.5. Physical evaluation of chloramphenicol in situ gel

2.5.1. Organoleptic

Organoleptic evaluation was done by looking at the *in situ* gel visually under a fluorescent lamp with a black and white background. Organoleptic evaluation was done by looking at the changes in the color, odor, and clarity of the preparation visually on the day of production and the 3^{rd}, 5^{th}, 7^{th}, 14^{th}, 21^{th}, and 28^{th} days of storage. It was expected that during the 28 storage days the preparation become colorless, clear, and odorless [26, 27].

2.5.2. pH

pH test was measured using a calibrated pH meter (Mettler-Toledo) at pH 4 and 7. The measurements of pH were carried out on the day of production, and on the 3^{rd}, 5^{th}, 7^{th}, 14^{th}, 21^{th} and 28^{th} days of storage at room temperature. The pH ranges expected to maintain stability of the *in situ* gel preparations are 5–7.4 [26,27].

Table 1. 3^2 factorial design optimization results of chloramphenicol ophthalmic *in situ* gel.

Chemicals	Formulas of Chloramphenicol *in situ* gel (% w/v)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Chloramphenicol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylenglycol	10	10	10	10	10	10	10	10	10
Poloxamer 407	5	7.5	10	5	7.5	10	5	7.5	10
HPMC	0.45	0.45	0.45	0.725	0.725	0.725	1	1	1
Benzalkonium chloride*	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Aqua pro injection q.s.	100	100	100	100	100	100	100	100	100

Note: F1 – F9 = Formula 1 – Formula 9
* Amount of Benzalkonium chloride in terms of % v/v.

2.5.3. Viscosity

Viscosity measurement was determined using a Rion VT-03F viscometer. Viscosity measurements were performed on the day of production, and the 3rd, 5th, 7th, 14th, 21th and 28th days of storage at room temperature. The viscosity range expected to extend the contact time of the formulation with ocular tissue in the eye is 5–100 cPs [26, 27].

2.5.4. Gelling capacity

In vitro gelling capacity of the formulation was determined by placing 2 mL of freshly prepared simulated tear fluid (STF) (35 °C ± 1 °C) in a vial. We accurately measured 20 μL of *in situ* gel formulation that was added to STF with mild agitation, which prevented the gel formation from breaking up. Gelling was observed visually. The time taken for its gelling and melting was noted [15, 27, 28]. Freshly prepared STF can be made with the composition in Table 2.

2.6. Data analysis

Data analysis was performed based on optimization results with a three-level factorial design of the response surface method. Organoleptic, pH, viscosity, and gelling capacity were evaluated for formula optimization, but only pH and viscosity that were for data analysis with factorial design, because they have an interval measurement scale, while organoleptic and gelling capacity were descriptively optimized. The results of physical evaluation results (pH and viscosity) were obtained from the 28 days of storage of the formulation. The software used to conduct data analysis was Design Expert® 11.

3. Results and discussion

The organoleptic and gelling capacity results of chloramphenicol ophthalmic *in situ* gel throughout 28 days of storage are shown in Table 3.

Table 3 shows that there was no organoleptic change such as color and clarity or aroma of the chloramphenicol *in situ* gel preparations. These results met the requirements of ophthalmic preparations [26]. Testing the capacity of the formation of the gel was carried out aimed to see the formation time of the sol to gel phase and the melting time of the preparation. The formula of F3, F6 and F9 showed the optimum time for gel formation and the longest time for melting. Whereas the formula of F1, F4 and F7 required the longest time for gel formation and the fastest time for melting.The optimum *in situ* gel is expected to be able to form a gel immediately and not long time of contact with ocular tissue, because long time of contact can cause uncomfartable use of the preparation for patients.

Table 2. The composition of simulated tear fluid (STF).

Chemicals	Amount
Sodium chloride (NaCl)	0.670 g
Sodium bicarbonate	0.2 g
Calcium chloride dihydrate	0.008 g
Purified water	ad 100 mL

The results of pH evaluation of ophthalmic *in situ* chloramphenicol gel within 28 days of storage are shown in Table 4.

Table 4 shows no significant change in all formulas at the pH value with the days of storage. All formulas met the pH requirements for ophthalmic gel preparations, from 5 to 7.4. Ideally, the pH of ophthalmic drops should be equivalent to that of tear fluid, which is 7.4. However, the value should be based on stability considerations. The pH selected should be the optimum for both stability of the active pharmaceutical ingredient and physiological tolerance [26, 27]. All formulas remain stable in terms of pH. The pH meter used has high accuracy with two digits, and the triplo measurements gave the average value that met the requirements.

The results of viscosity evaluation of chloramphenicol ophthalmic *in situ* gel within 28 days of storage are shown in Table 5.

Table 5 shows that the viscosity of chloramphenicol ophthalmic *in situ* gel was tested within 28 days of storage at 25 ± 2 °C (temperature control at room testing). Viscosity results showed that F3, F6, F8, F9 met the viscosity requirements of ophthalmic *in situ* gel viscosity, 5–100 cPs [26, 27]. These formulas could increase the contact time of the preparation with ocular tissue in the eye. A Rion VT-03F viscometer (with a dial gauge) has a measurement accuracy within 5% of scale maximum. The average value of viscosity met the requirements probably because the measurements were carried out in triplo. The choice of storage time was based on the stability time of preparations made in the laboratory scale. But in industry, stability testing should be carried out for 3–6 months using the accelerated method.

Data analysis using a three-level factorial design method with Design Expert® 11 software was carried out to find the formula that produced the most optimum response value. The response values tested in the software are pH and viscosity. Then, the response value that will be entered into this software will show the most influencing factors on the intended response value and determine study on the interactions between factors that affect the response value. The organoleptic and gelling capacity data used for the requirements of ophthalmic preparations [26]. Testing the gelling capacity was carried out aimed to see the formation time of the sol to gel phase and the melting time of the preparation. The optimum *in situ* gel is expected to be able to form a gel immediately and not long time of contact with ocular tissue, because long time of contact can cause uncomfartable use of the preparation for patients.

The first analysis was to examine the correlation value (degree of relationship) of poloxamer 407 and HPMC with pH and viscosity. The results of the correlation analysis of poloxamer 407 and HPMC concentration against pH are shown in Figure 1.

Based on Figure 1, the correlation value between poloxamer 407 concentration and pH was 0.004, while the correlation value between HPMC concentration and pH was 0.075. The both correlation values were included in the category of negligible correlations [29]. Meanwhile, the results of the correlation analysis of poloxamer 407 and HPMC concentration on viscosity are shown in Figure 2.

Table 3. Organoleptic and gelling capacity of chloramphenicol ophthalmic *in situ* gel on the 28 days of storage.

Formula	Results from the 28 days of storage	
	Organoleptic	Gelling Capacity
F1	Odorless, no changes in color and clear	+
F2	Odorless, no changes in color and clear	++
F3	Odorless, no changes in color and clear	+++
F4	Odorless, no changes in color and clear	+
F5	Odorless, no changes in color and clear	++
F6	Odorless, no changes in color and clear	+++
F7	Odorless, no changes in color and clear	+
F8	Odorless, no changes in color and clear	++
F9	Odorless, no changes in color and clear	+++

Notes: + = gel is formed in more than 40 s and melts in 1–2 min, ++ = gel is formed within 30–40 s and melts in 2–5 min, +++ = gel is formed in less than 30 s and melts in more than 5 min.

Table 4. pH of chloramphenicol ophthalmic *in situ* gel within 28 days of storage.

Formula	pH of formula at days of storage						
	0	3	5	7	14	21	28 (day)
F1	6.74 ± 0.01	6.66 ± 0.01	6.67 ± 0.01	6.69 ± 0.02	5.87 ± 0.01	5.71 ± 0.01	5.78 ± 0.01
F2	6.74 ± 0.00	6.81 ± 0.02	6.79 ± 0.01	6.57 ± 0.03	6.55 ± 0.03	6.56 ± 0.00	6.34 ± 0.02
F3	6.77 ± 0.01	6.81 ± 0.01	6.8 ± 0.01	6.81 ± 0.02	6.33 ± 0.01	6.53 ± 0.02	5.66 ± 0.02
F4	6.70 ± 0.01	6.78 ± 0.01	6.76 ± 0.02	6.57 ± 0.03	6.55 ± 0.03	6.60 ± 0.00	6.52 ± 0.01
F5	6.77 ± 0.02	6.75 ± 0.02	6.74 ± 0.01	6.69 ± 0.02	6.75 ± 0.00	6.62 ± 0.01	6.51 ± 0.01
F6	6.84 ± 0.01	6.84 ± 0.01	6.83 ± 0.01	6.85 ± 0.02	6.69 ± 0.01	6.69 ± 0.02	6.22 ± 0.02
F7	6.66 ± 0.06	6.63 ± 0.04	6.76 ± 0.02	6.63 ± 0.02	5.96 ± 0.03	5.80 ± 0.02	5.63 ± 0.02
F8	6.87 ± 0.01	6.90 ± 0.01	6.83 ± 0.01	6.85 ± 0.02	6.69 ± 0.01	6.51 ± 0.01	6.27 ± 0.01
F9	6.78 ± 0.01	6.79 ± 0.02	6.79 ± 0.01	6.81 ± 0.01	6.48 ± 0.01	6.20 ± 0.01	6.06 ± 0.01

Table 5. Viscosity of chloramphenicol ophthalmic *in situ* gel within 28 days of storage.

Formula	Viscosity of formula at days of storage (cPs)						
	0	3	5	7	14	21	28 (day)
F1	2 ± 0.00	1.75 ± 0.00	2 ± 0.00	2 ± 0.00	2 ± 0.00	1.58 ± 0.14	1.83 ± 0.14
F2	4 ± 0.00	3 ± 0.00	3 ± 0.00	2.17 ± 0.29	2 ± 0.00	2 ± 0.00	2.33 ± 0.29
F3	3.67 ± 0.29	5 ± 0.00	5 ± 0.00	5 ± 0.00	5 ± 0.00	5 ± 0.00	5 ± 0.00
F4	2.50 ± 0.00	2 ± 0.00	2.50 ± 0.00	2.17 ± 0.29	2 ± 0.00	2 ± 0.00	2 ± 0.00
F5	3 ± 0.00	2.50 ± 0.00	2 ± 0.00	2 ± 0.00	2 ± 0.00	2 ± 0.00	2.83 ± 0.29
F6	3.67 ± 0.29	5 ± 0.00	5 ± 0.00	5 ± 0.00	5 ± 0.00	6 ± 0.00	10 ± 0.00
F7	2 ± 0.00	1.75 ± 0.00	1.75 ± 0.00	1.75 ± 0.00	1.75 ± 0.00	1.50 ± 0.00	1.50 ± 0.00
F8	6.67 ± 0.58	6 ± 0.00	4 ± 0.00	4.33 ± 0.58	6 ± 0.00	4 ± 0.00	5 ± 0.00
F9	5.33 ± 0.58	15.33 ± 0.58	14 ± 0.00	11.67 ± 0.58	15 ± 0.00	13.33 ± 0.58	14.67 ± 0.58

Based on Figure 2 the correlation value between poloxamer 407 concentration and viscosity was 0.782 indicating a high degree of association. The correlation value between HPMC concentration and viscosity was 0.386 which shows a low correlation [29].

The next analysis is the selection for the best regression model of each physical parameter (pH and viscosity). The parameters for the recommended regression model given by ANOVA for pH response are shown in Table 6.

According to data in Table 6, the selection of the best regression model can be seen from the *p*-value generated by the model [30]. A *p*-value of less than 0.05 ($p < 0.05$) indicates that the recommended model is good. Based on Table 6, none of the *p*-values shown by any of the models is less than 0.05. Therefore, the *p*-value close to 0.05 is the quadratic model. The next process of selecting the best regression model for pH response was done by looking at Table 7.

The parameters that must be considered for choosing the best regression model were found by looking at the lowest standard deviation, the largest determinant coefficient (R^2), R^2_{adj}, and R^2_{pred}, and the lowest PRESS value [31]. Table 7 indicates that the equation model that met those requirements is the quadratic model. The parameter for the recommended regression model given by ANOVA for viscosity response are shown in Table 8.

In Table 8, it is shown that the lowest p-value was found in the quadratic model. Based on Table 9, the regression model that has the lowest standard deviation and the highest R^2 and R^2_{adj} are in the cubic equation model, but the highest R^2_{pred} and lowest PRESS were generated by the quadratic equation. Therefore, the recommended regression equation is the quadratic model.

ANOVA statistical analysis on data in Table 9 gave the recommended formula with the best poloxamer 407 and HPMC composition (F_{pred}).

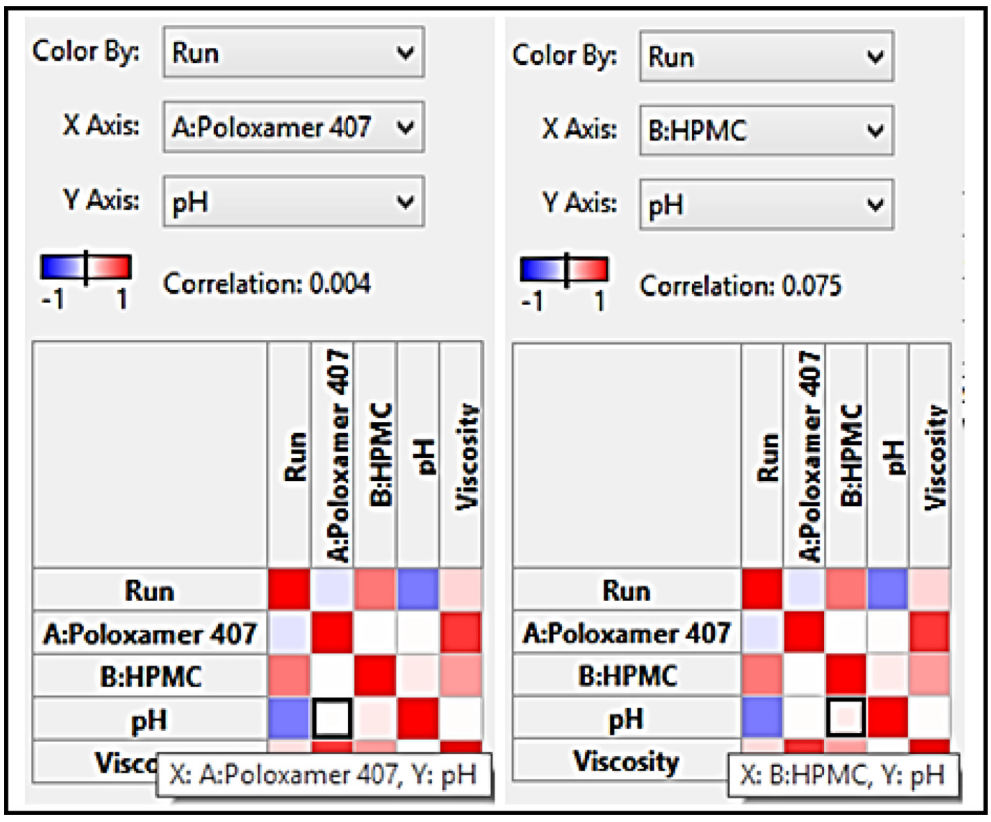


Figure 1. Correlation of poloxamer 407 and HPMC with pH.

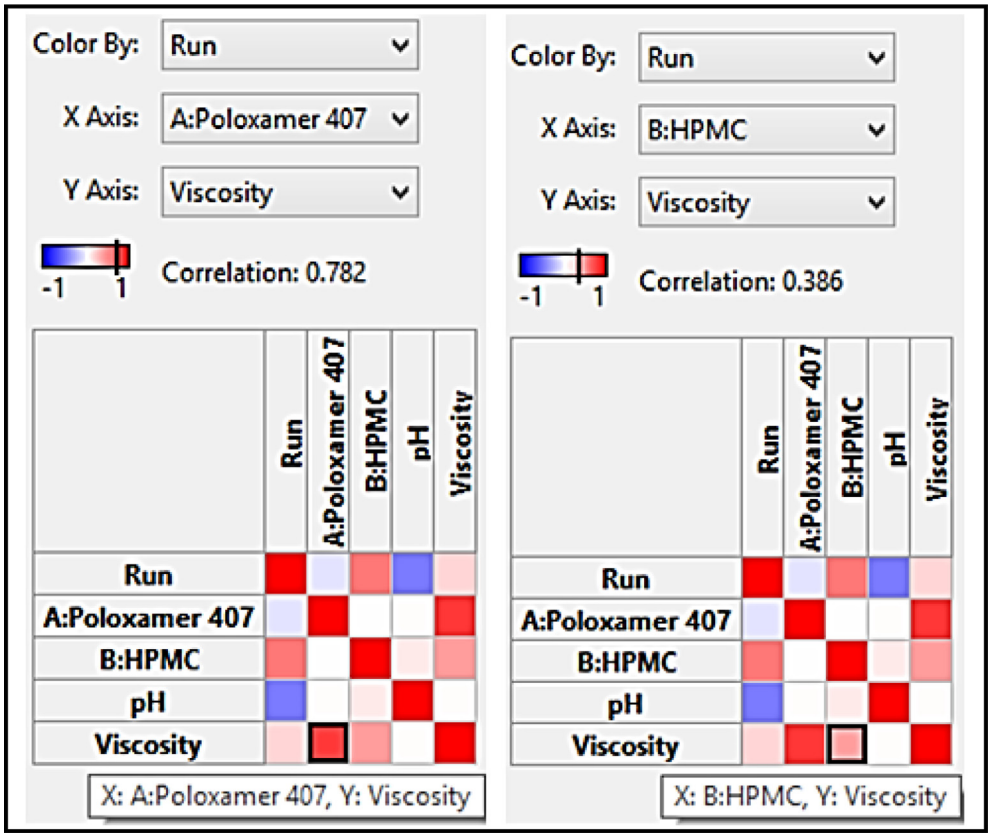


Figure 2. Correlation of poloxamer 407 and HPMC concentrations to viscosity.

Table 6. Analysis of variance (ANOVA) each regression model for pH response.

Source	Sum of squares			Df			F-value	p-value*
	Regression	Residual	Total	Regression	Residual	Total		
Linear	0.0054	0.9576	0.9630	2	6	8	0.0170	0.9832
2FI	0.0810	0.8820	0.9630	3	5	8	0.1531	0.9223
Quadratic	0.8163	0.1467	0.9630	5	3	8	3.3400	0.1750
Cubic	0.8980	0.0650	0.9630	7	1	8	1.9700	0.5005

* *p*-value is set at ($\alpha = 0.05$).

Table 7. Model summary statistics for pH response.

Source	Standard Deviation	R^2	R^2_{adj}	R^2_{pred}	PRESS	Notes
Linear	0.3995	0.0056	-0.33	-1.5287	2.44	-
2FI	0.4200	0.0842	-0.4654	-4.8138	5.6	-
Quadratic	0.2211	0.8477	0.5937	-0.5364	1.48	Suggested
Cubic	0.2550	0.9325	0.4598	-11.3061	11.85	Alias

Table 8. Analysis of variance (ANOVA) each regression model for viscosity response.

Source	Sum of Squares			df			F-value	p-value*
	Regression	Residual	Total	Regression	Residual	Total		
Linear	122.78	38.86	161.64	2	6	8	9.48	0.0139
2FI	147.78	13.86	161.64	3	5	8	17.77	0.0043
Quadratic	159.78	1.87	161.64	5	3	8	51.37	0.0042
Cubic	161.12	0.5232	161.64	7	1	8	43.99	0.1156

* *p*-value is set at ($\alpha = 0.05$).

This software also provided the pH and viscosity value in 3-dimensional response surface plots, contour plots and regression equation for all formulas (F1–F9 and F_{pred}). The 3-dimensional response surface plot and the contour plot that illustrated the relationship between pH with poloxamer 407 and HPMC concentrations are shown in Figure 3.

Figure 3(a) shows that the equation model that illustrates the relationship of pH with the concentration of poloxamer 407 and HPMC is a quadratic equation model that has a maximum pH point. From Figure 3(b), shows the map poloxamer 407 on the x-axis, HPMC on the y-axis, and the pH value as a contour. pH value will increase if the concentration of poloxamer 407 and HPMC used approaches the red area, because in the red area the highest pH value is generated. Formula recommended by Design Expert® 11 (Fpred) software will be predicted to have a pH of 6.65.

The quadratic equation model that illustrates the relationship between pH and poloxamer 407 and HPMC concentrations was:

$$pH = 0.93 + 0.803A + 7.43B + 0.2AB - 0.06A^2 - 6.08B^2$$

Notes:

A = poloxamer 407 concentration,
B = HPMC concentration.

The 3-dimensional response surface plot and the contour plot that illustrated the relationship between viscosity and poloxamer 407 and HPMC concentrations were shown in Figure 4.

From Figure 4, when viewed from a 3-dimensional and the contour plots of surface viscosity responses, the equation model that illustrates the relationship of viscosity with the concentration of poloxamer 407 and HPMC is a quadratic equation model with a minimum viscosity point. The contour plot graph of viscosity response will map poloxamer 407 on the x axis, HPMC on the y axis, and viscosity value as contour. The viscosity response control chart shows that the viscosity value will increase if the concentration of poloxamer 407 and HPMC used approaches the red area, because in the red area the highest viscosity value is produced. Formula recommended by Design Expert® 11 (Fred) software will be predicted to have a viscosity value of 5 cPs.

The quadratic equation model that illustrated the relationship between viscosity and poloxamer 407 and HPMC concentrations is

$$\text{Viscosity} = 28.43–6.88A - 22.13B + 3.64AB + 0.39A^2 + 1.48B^2$$

Notes: A = poloxamer 407 concentration,

B = HPMC concentration.

Response surface methodology (RSM) will provide the best poloxamer 407 and HPMC composition solutions based on the physical parameters (F_{pred}). After determining the pH and viscosity requirements on the ophthalmic *in situ* gel, this software will provide a solution based on desirability value. The pH, viscosity, and desirability value of F1–F9 and F_{pred} were given in Table 10.

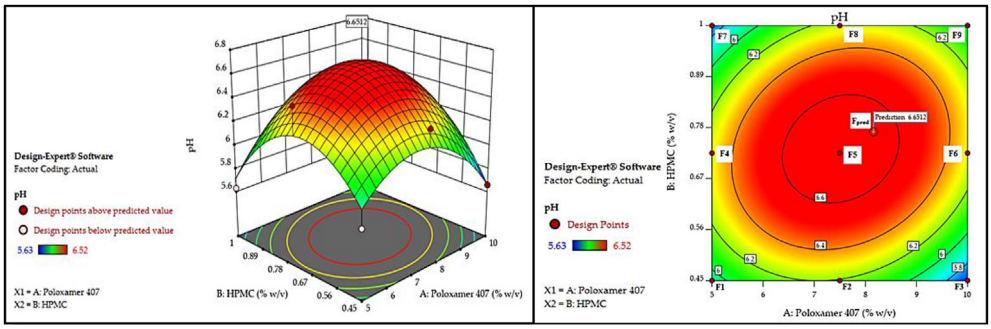
Based on Table 10, it can be seen that from the all formulas, F6 had the highest desirability value of 0.54. The recommended formula given by Design Expert® 11 software (F_{pred}) was the formula of poloxamer 407 8.16% *w/v* and HPMC 0.77% *w/v* with a desirability value of 0.69. The 3-dimensional response surface and contour plot result based on desirability value was shown in Figure 5.

Figure 5(a) shows that desirability value explains the closeness of a response value generated by the factor (X_1 and X_2) to the desired requirements value (pH and viscosity). The desirability value is in the range of 0–1. The closer the desirability value to 1, the better the software's ability to produce optimum formulas [32, 33].

Contour plot graph in Figure 5(b) shows that the prediction formula recommended by the software (Fpred) shows gives a pretty good

Table 9. Model summary statistics for viscosity response.

Source	Standard Deviation	R^2	R^2_{adj}	R^2_{pred}	PRESS	Notes
Linear	2.55	0.7596	0.6794	0.3325	107.89	-
2FI	1.67	0.9142	0.8628	0.7138	46.26	-
Quadratic	0.7887	0.9885	0.9692	0.8746	20.26	Suggested
Cubic	0.7233	0.9968	0.9741	0.4101	95.36	Alias

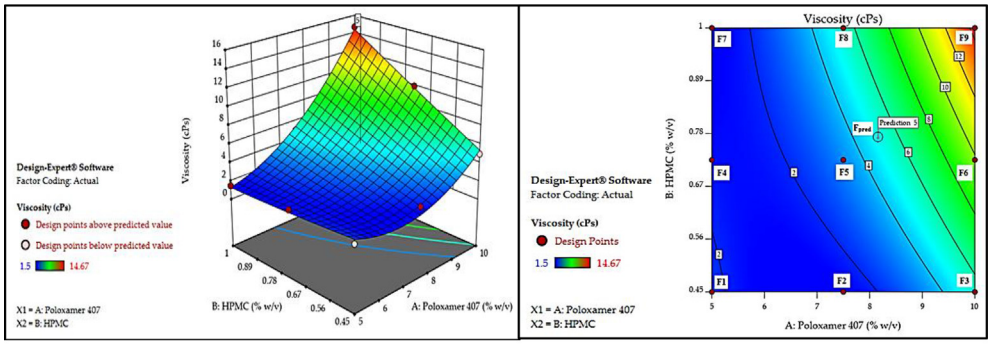


(a) (b)

Figure 3. The 3-dimensional response surface plot (a) and the contour plot for pH response (b).

response with the desirability value ranging from 0 to 1. If the response approaches the blue contour, the resulting response does not meet the optimum pH and viscosity satisfaction criteria because the desirability value is close to 0. If the response approaches the red contour, the resulting response will increasingly meet satisfactory optimum pH and viscosity criteria (will increase satisfactory optimum of pH and viscosity criteria) because they are close to desirability value 1. The results given by Design Expert® 11 in this study are in the green area. with the desirability value of 0.69. It could be interpreted that the prediction value was better enough which indicates that the observed value was 0.69.

The optimal formulation results that obtained from this study more in-depth than some similar studies. For example, the research conducted by Rathod et al., only shows directly the results of factorial design optimization without complete explanation in determining the conclusion [34]. So with research conducted by Ashir et al., the factorial design just

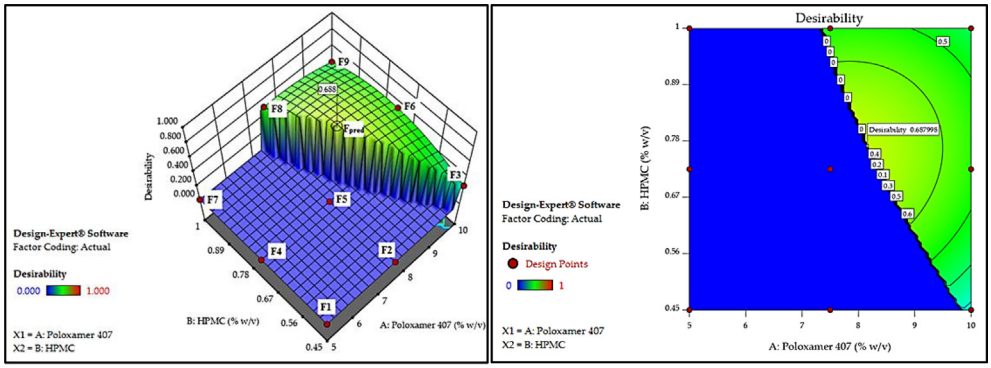


(a) (b)

Figure 4. The 3-dimensional plot (a) and the contour plot for viscosity response (b).

Table 10. The pH, viscosity, and desirability value of formula F1–F9 and F_{pred}.

Formula	Poloxamer 407 (% w/v)	HPMC (% w/v)	pH	Viscosity	Desirability
F1	5	0.45	5.78	1.83	0
F2	7.5	0.45	6.34	2.33	0
F3	10	0.45	5.66	5	0.27
F4	5	0.725	6.52	2	0
F5	7.5	0.725	6.51	2.83	0
F6	10	0.725	6.22	10	0.54
F7	5	1	5.63	1.5	0
F8	7.5	1	6.27	5	0.52
F9	10	1	6.06	14.67	0.41
F_{pred}	8.16	0.77	6.65	5	0.69



(a) (b)

Figure 5. (a) 3-dimensional response surface plot; (b) contour plot for desirability value.

explain 3D graphs, counter plot without desirability value and and higher use of poloxamer and HPMC bases than our study [32]. As a follow-up to this study, the further research is being conducted to optimize the formulations in terms of chemistry, microbiology, in vitro and in vivo studies as well as testing the safety of preparations.

4. Conclusions

The formula of chloramphenicol *in situ* gel could be optimized using factorial design based on its physical parameters including organoleptic properties, gelling capacity, pH and viscosity. All formulas have met the Indonesian pharmacopoeia requirements according to the physical evaluation. Formula 6 (F6) was the best formula with the highest desirability value of 0.54 which was supported by the result of factorial design analysis.

5. Patents

There is no patent resulting from the work reported in this manuscript.

Declarations

Author contribution statement

I.S. Kurniawansyah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

T. Rusdiana and H.A. Wahab: Conceived and designed the experiments; Wrote the paper.

I. Sopyan: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

H. Ramoko: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

A. Subarnas: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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