International Journal of Applied Pharmaceutics
I. S. K
ISSN- 0975-7058 Vol 13, Special Issue 4, 2021

Original Article

IN VITRO DRUG RELEASE STUDY OF CHLORAMPHENICOL *IN SITU* GEL WITH BASES MIXTURE OF POLOXAMER 407 AND HPMC BY OPTIMIZATION WITH FACTORIAL DESIGN

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Received: 08 Aug 2021, Revised and Accepted: 10 Sep 2021

ABSTRACT

Objective: The objective of this study was to find the best base mixture composition (poloxamer 407 and HPMC) of chloramphenicol *in situ* gel formula based on *in vitro* property (Cumulative amount of drug release).

Methods: The *in vitro* diffusion of chloramphenicol *in situ* gel study was carried out using franz diffusion cells to know the effect of the Critical Process Parameters (CPPs) as independent variables (poloxamer 407 and hydroxypropyl methylcellulose (HPMC)) on the Critical Quality Attribute (CQA) as dependent variable (cumulative amount of drug release) with 2^2 factorial design.

Results: 2^2 factorial design of chloramphenicol *in situ* gel yielded 4 variations of poloxamer 407 and HPMC bases component in %w/v as follows, F1 (5:0.45), F2 (10:0.45) F3 (5:1) and F4 (10:1). The amount of drug release results from *in vitro* dissolution assay were 30.60% (F1), 45.64% (F2), 58.30% (F3), and 22.50%) (F4).

Conclusion: Formula 3 (F3) was considered as the best formula component in terms of *in vitro* assay of chloramphenicol *in situ* gel with a desirability value of 0.58.

Keywords: Chloramphenicol, *In situ gel, In vitro* diffusion, Poloxamer 407, HPMC, Franz diffusion, Factorial design, Critical process parameters, Critical quality attribute

DOI: https://dx.doi.org/10.22159/ijap.2021.v13s4.43829 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Conventional ophthalmic solutions are often rapidly eliminated after administration and usually cannot provide and maintain sufficient drug concentrations in the pre-corneal region. Therefore, conventional ophthalmic solutions often provide low levels of bioavailability in the eye [1–4]. One of the efforts to solve this problem is to extend the contact time of ophthalmic formulations with ocular tissues by increasing the viscosity of the formulation with polymeric hydrogels [5–8]. The use of bioadhesive polymers result in an increase of ocular residence time via their enhanced viscosity and mucoadhesive properties. Given that the increase in viscosity of ophthalmic formulations frequently causes blurred vision, it is important to achieve the optimum viscosity range and the most proper rheological behavior to ensure good efficacy and tolerance [4, 6, 7, 9].

To cope with these drawbacks, *in situ* gel systems have emerged as one of the best novel drug delivery systems. It can enhance the bioavailability of ocular drugs by sustaining and controlling the release of drugs in the pre-corneal region [10, 11]. *In situ* drug delivery systems consist of polymers that develop sol-to-gel phase transition due to changes such as temperature, pH or electrolyte composition of the eye environment [12, 13]. As an ocular drug delivery system, *in situ* gel can be instilled as drops into the cul-de-sac of the eye and get transformed into a gel [14].

One of the thermoresponsive polymers that is commonly used for *in situ* drug delivery systems is poloxamer 407 (Pluronic F-127). It could sustain the release of drugs in the eye tissue [15–17]. When used itself, poloxamer 407 has poor mucoadhesive nature. Adding an excessive amount of poloxamer 407 could also trigger the risk of hypertriglyceridemia in the eye, which causes blurry vision. Therefore, it could be fixed by enhancing it with hydroxypropyl methylcellulose (HPMC) as a viscosity-enhancing agent [15, 16, 18]. The addition of HPMC could make better mucoadhesive nature and decrease the amount of poloxamer 407 needed to form *in situ* gel, so it did not cause blurry vision [19].

Design of Experiments (DoE) is a powerful and efficient tool for optimizing pharmaceutical formulations. It is one of several Quality by Design (QbD) development phases. When developing the quality of the product, various techniques of DoE can be used for it [20, 21]. Optimization by DoE refers to the process of planning the experiment in a systematic way so suitable datas can be collected and analyzed statistically, resulting in a valid and objective conclusion [22, 23]. It can be used to determine which Critical Process Parameter(s) (CPP(s)) is the most important to the Critical Quality Attribute(s) (CQA(s)). One of the DoE that was recently used for this satisfaction need is Factorial Design. This DoE allows the effect of several factors, even interactions between them to be estimated with the same number of trials as are necessary to odetermine the single effect itself with the same degree. Factorial design is favourable when examining treatment variations, combining independent studies into one and examining interaction effects between the factors [24]. This design can be used as a preliminary design for more complex response surface design modeling [25, 26].

This research came with the aim of finding the best bases mixture composition (poloxamer 407 and HPMC) of chloramphenicol *in situ* gel formula. It could be achieved by optimizing the poloxamer 407 and HPMC with 2-level factorial design. On top of that, this design could determine the mathematical relationship between the CQA (Cumulative amount of drug release) and the CPPs (poloxamer 407 and HPMC) with a polynomial equation. The optimized formulas were evaluated based on their *in vitro* property (Cumulative amount of drug release) for data analysis with 2^2 factorial design.

MATERIALS AND METHODS

Chemicals

The chemicals used were chloramphenicol antibiotic (Bio Basic Inc., Markham Ontario, Canada), poloxamer 407 (Kolliphor®P 407, BASF Indonesia), hydroxypropyl methylcellulose (HPMC) (Colorcon®,

Indonesia), 70% ethanol (Ikapharmindo Putramas, Indonesia), 96% ethanol *pro analysis* (Brataco®, Indonesia), benzalkonium chloride (Merck, Indonesia), propylene glycol (Ikapharmindo Putramas, Indonesia), aqua pro injection (Ikapharmindo Putramas, Indonesia), sodium chloride (Merck, Indonesia), calcium chloride dihydrate (Merck, Indonesia), sodium bicarbonate (Merck, Indonesia).

Formulation of *in situ* gel chloramphenicol formula with 2^2 factorial design

Optimization of the formula was done by using 2^2 factorial design. Poloxamer 407 (A) and HPMC (B) in terms of %w/v concentration were selected as the independent variables (factors), while % of the cumulative amount of drug release was selected as the dependent variable (response). The actual values at lower and upper level for (A) were 5% and 10%, while actual values at lower and upper lever for (B) were 0.45% and 1%. The formula(s) resulted from 2^2 factorial design generated 4 variations of formula, which was shown in table 1. Each formula was made according to the procedure and was examined for the *in vitro* evaluation (% of cumulative drug release) after the formula had been produced.

Formulation procedure of *in situ* gel chloramphenicol formula

Each formula was made aseptically under a laminar airflow (LAF) room which had been sterilized before with 70% alcohol. After sterilization, the ultraviolet (UV) lamp was turned on for 1.5 hour. Then, the working light and the blower were turned on.

The formulation process starts with dissolved chloramphenicol in propylene glycol. After completely dissolved, benzalkonium chloride was added. The chloramphenicol mixture was stirred and separated. Then, each poloxamer 407 or HPMC was dissolved with aqua pro injection on a separated container until dissolved completely. And then, poloxamer 407 and HPMC were mixed together in one container as *in situ* gel base mixture. This mixture was sterilized with an autoclave at 121 °C for 15 min. This autoclave-sterilized base mixture was added with the chloramphenicol mixture that had been sterilized before with 0.2 µm bacterial filter until homogenous and cooled down [27].

Table 1: 2^2 factorial design results of *in situ* gel chloramphenicol formulation

Chemical(s)	Formula composition of *in situ* gel chloramphenicol in terms of %w/v			
	F1	F2	F3	F4
Chloramphenicol	0.5	0.5	0.5	0.5
Propylenglycol	10	10	10	10
Poloxamer 407	5	10	5	10
HPMC	0.45	0.45	1	1
Benzalkonium chloride	0.01	0.01	0.01	0.01
Aqua pro injection q. s.	100	100	100	100

Calibration curve of chloramphenicol

The chloramphenicol calibration curve was made by dissolving 100 mg of chloramphenicol with ethanol (q. s.) until completely dissolved. Then it was added with aqua pro injection until 100 ml for 1000 ppm of chloramphenicol stock solution. Five concentrations of chloramphenicol from 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm were created by diluting chloramphenicol stock solution (1000 ppm). The absorbance of each concentration was examined with a UV-Vis spectrophotometer at 280 nm wavelength [28].

***In vitro* diffusion assay of chloramphenicol *in situ* gel**

The *in vitro* drug release assay was studied by using a *franz* diffusion cell. This assay was performed by injecting 3 ml of chloramphenicol *in situ* gel formulation on donor compartment and 13 ml of simulated tear fluid (STF) at pH 7.4±0.2 on acceptor compartment. Cellophane membrane was swelled under STF and was bounded between the end of both compartments on the *franz* diffusion cell, and the rotation speed was set at 50 rpm. The STF temperature was maintained at 37±0.5 °C using a magnetic stirrer [16, 29–31].

This assay was done by taking 1 ml of the sample at a certain period of time; 5, 15, 30, 45, 60, 120, 180 until 480 min. Each sampling was taken 3 times. When the sample was taken at a certain volume, this taken volume was replaced with the same amount of that volume [16,31,32]. Each sample (at a certain interval of time) was analyzed by using UV-Vis spectrophotometer at 280 nm wavelength aided with the blank solution [32]. The drug content was calculated by using calibration curve regression.

Determination of *in vitro* kinetic release [33, 34]

The *in vitro* kinetics release of chloramphenicol *in situ* gel was analyzed by using zero-order model, first-order model, Higuchi model and Korsmeyer-Peppas model [33]. Each *in vitro* kinetics model was determined by plotting the y-axis and x-axis as shown in table 2.

Table 2: *In vitro* kinetic release model plotting

In vitro kinetic model	Y-axis	X-axis
Zero-order	Cumulative amount of drug release	Time
First-order	Log(Cumulative amount of drug release)	Time
Higuchi	Cumulative amount of drug release	$\sqrt[2]{time}$
Korsmeyer-Peppas	Log(Cumulative amount of drug release)	Log(Time)

Korsmeyer-Peppas equation had a concern at the *n* value (release exponent) that describes the mechanism release of drug substance. The mechanism release of drug parameters was shown on table 3.

Table 3: Korsmeyer-peppas drug release mechanism parameters

n (release exponent)	Mechanism release
$n<0.45$	Fick Diffusion
$0.45<n<0.89$	Non-Fickian Diffusion
$n=0.89$	Case Transport II
$n>0.89$	Supercase Transport II

Data analysis

Data analysis was conducted with 2^2 factorial design. Percent cumulative (%) of drug substance release was evaluated for formula optimization, by using *Design Expert*®11 software (trial version). This software could analyze the data with an interval measurement scale. This analysis can find the formula with the most desirable response value (cumulative amount of drug release) and show the most important factors on the intended response. The results of *in vitro* drug release (Cumulative amount of drug release) data were obtained after 8 h of sampling and analyzed using Analysis of variance (ANOVA). p-value is set at ($\alpha = 0.05$).

RESULTS

The absorbance results of chloramphenicol at various concentrations on parts per million (ppm) were shown on table 4. Regression result of the chloramphenicol calibration curve is y = 0.025x+0.0254.

Table 4: The absorbance results for chloramphenicol calibration curve

Concentration (ppm)	Absorbance
10	0.2792
15	0.4018
20	0.5193
25	0.6500
30	0.7813

The *in vitro* diffusion assay was done by using the principle of *franz* diffusion cells. Sample was placed on the membrane that was stretched on the tube to form donor and acceptor compartment. The *in vitro* drug release results of chloramphenicol *in situ* gel formula after 8 h were shown on fig. 1.

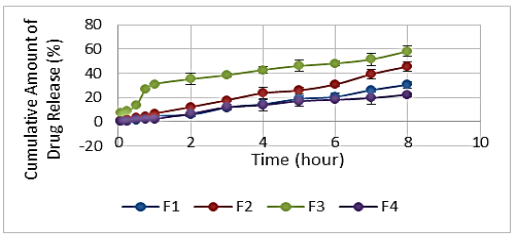


Fig. 1: The release profile of chloramphenicol *in situ* gel (n=3)

From the obtained data, the chloramphenicol *in situ* gel rate of release for F1 = 0.038 (mg/l) h^{-1}, F2 = 0.056 (mg/l) h^{-1}, F3 = 0.064 (mg/l) h^{-1}, dan F4 = 0.027 (mg/l) h^{-1}. The *in vitro* kinetic release of chloramphenicol *in situ* gel was shown on table 5.

The first analysis was to analyze the half-normal plot. This plot can be used to evaluate the most important factor according to the cumulative amount of drug release response. Fig. 2 showed the half-normal plot between poloxamer 407, HPMC and combination of these excipients analysis result

Table 5: The *in vitro* kinetic release of chloramphenicol *in situ* gel

Formula	Zero-order	First-order	Higuchi	Korsmeyer-Peppas		Transport mechanism
	r^2	r^2	r^2	r^2	n	
F1	0.9859	0.7180	0.9297	0.9751	1.1826	*Supercase II*
F2	0.9912	0.8448	0.9393	0.9471	1.0883	*Supercase II*
F3	0.8213	0.5097	0.9544	0.7433	0.9692	*Non fickian*
F4	0.9642	0.7952	0.9591	0.9829	1.0815	*Supercase II*

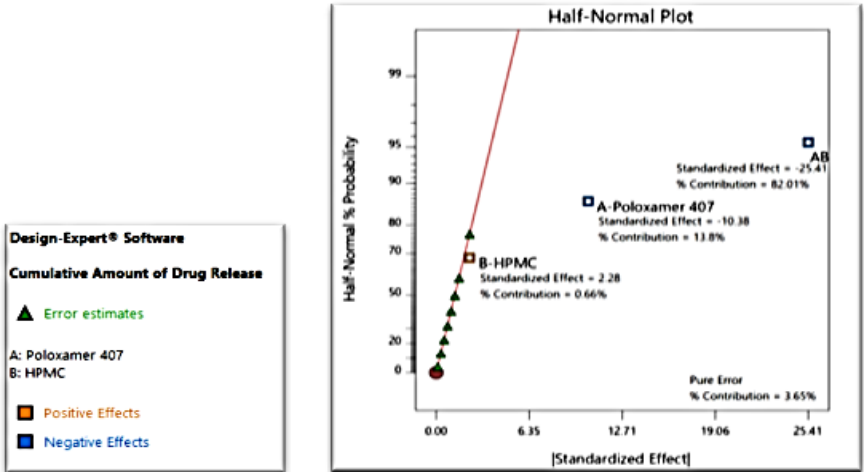


Fig. 2: The half-normal plot to cumulative amount of drug release with the same standard deviation
Note: AB = Combination of poloxamer 407 and HPMC

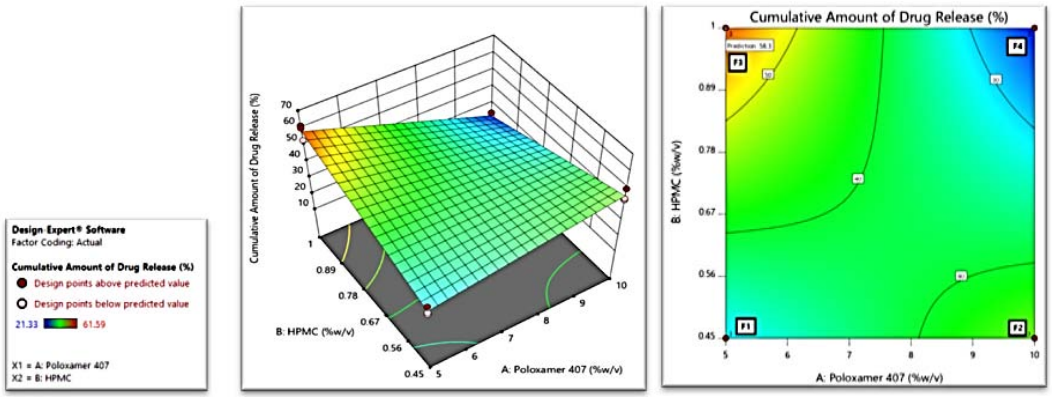
The combination of poloxamer 407 and HPMC was considered as the most important factors related to cumulative amount of drug release. Analysis of variance (ANOVA) for the selected response (Cumulative amount of drug release) model was shown on table 6. Since 2-level full factorials are only used for screening design, the only available ANOVA regression model follows the 2 Factors of Interaction (2FI) model.

Table 6: Cumulative amount of drug release response in the form of 2FI model

Source	Sum of squares	df	Mean square	F-value	*p-value	Notes
Model	2276.72	3	758.91	70.45	<0.0001	Significant
A-Poloxamer 407	323.34	1	323.34	30.02	0.0006	
B-HPMC	15.62	1	15.62	1.45	0.263	
AB	1937.77	1	1937.77	179.89	<0.0001	
Pure Error	86.17	8	10.77			
Corr Total	2362.9	11				

*p-value was set at (α = 0.05).

The relationship in the form of 2FI regression between cumulative amount of drug release, poloxamer 407 and HPMC was explained by the response surface plot shown on fig. 3 and contour plot shown on fig. 4.



(a) **(b)**

Fig. 3: The response surface (a) and The contour (b) plot profile (2FI) for the cumulative amount of drug release of chloramphenicol *in situ* gel, Standard error analysis was used to examine the design accuracy and precision. The 2-level full factorial design contour plot of standard error is shown on fig. 4

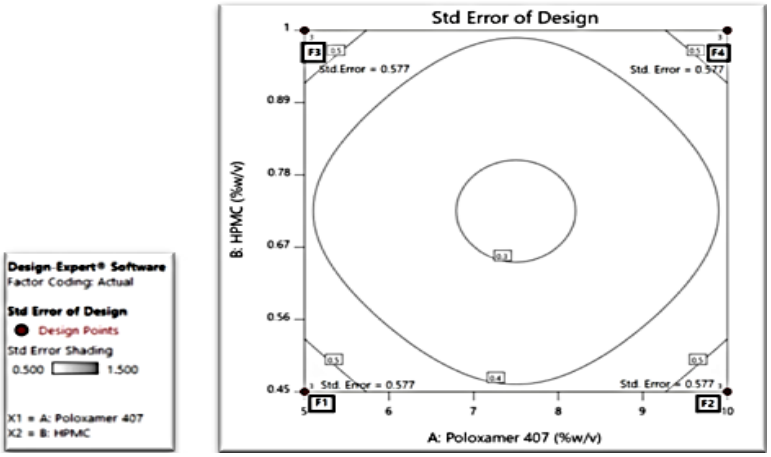
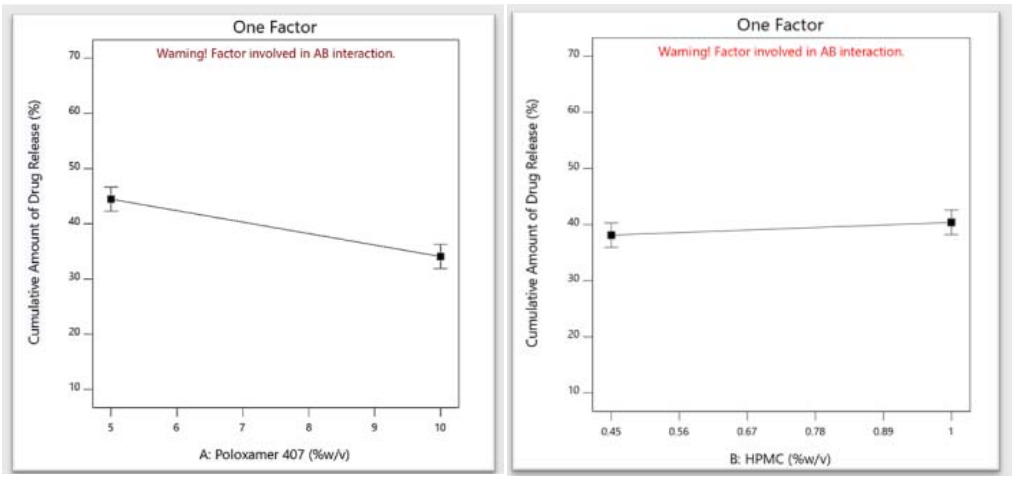


Fig. 4: Standard error analysis of two-level full factorial design between Poloxamer 407 and HPMC

Graphical effect of main factors (poloxamer 407 or HPMC) and its interaction to the cumulative amount of drug release response is shown on fig. 5 and 6. The effect between poloxamer 407/HPMC and the cumulative amount of drug release is shown at fig. 5.



(a) **(b)**

Fig. 5: Effect of poloxamer 407 (a) and HPMC (b) concentration to the cumulative amount of drug release

It had been proven with the ANOVA analysis from the *p*-value on table 6. Graphical effect between poloxamer 407 and HPMC concentration and the cumulative amount of drug release is shown at fig. 6**.**

The software would recommend a solution for the best composition of poloxamer 407 and HPMC based on the cumulative amount of drug release by utilizing desirability value. Table 7 showed the best formula composition, which could be seen from the cumulative amount of drug release and desirability value.

The desirability value contour plot is shown on fig. 7(a) and the response surface plot is shown on fig. 7(b) for poloxamer 407 and HPMC concentration.

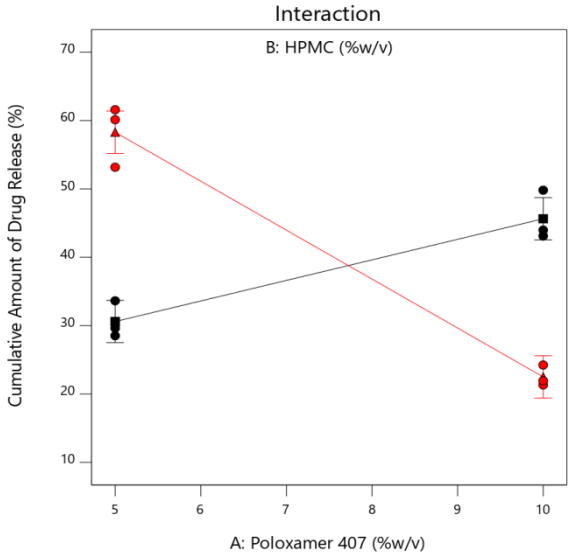
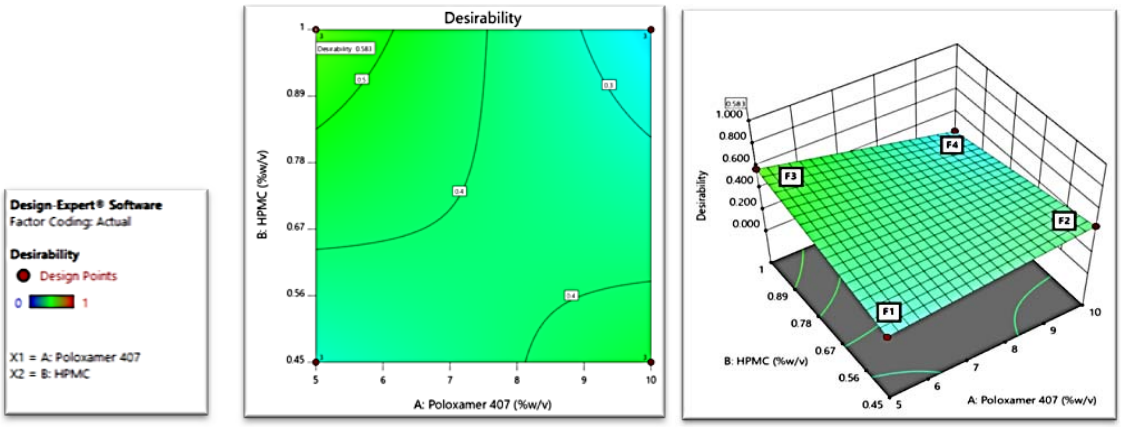


Fig. 6: Effect of poloxamer 407 and HPMC concentration to the cumulative amount of drug release
Notes: Red line: HPMC concentration at 1%, Black line: HPMC concentration at 0.45%

Table 7: The cumulative amount of drug release (%) and the desirability value of the best poloxamer 407 and HPMC composition of chloramphenicol *in situ* gel

Formula	Poloxamer 407 (%w/v)	HPMC (%w/v)	Cumulative amount of drug release (%)	Desirability	Notes
F1	5	0.45	30.60	0.31	
F2	10	0.45	45.64	0.46	Recommended
F3	5	1	58.30	0.58	Recommended
F4	10	1	22.50	0.22	
F_{pred}	5.56	1	54.32	0.54	Recommended



(a) **(b)**

Fig. 7: The contour (a) and the response surface (b) plot for desirability value

DISCUSSION

Regression result of the chloramphenicol calibration curve is $y = 0.025x + 0.0254$. The *in vitro* diffusion assay was done by using the principle of *franz* diffusion cells. Sample was placed on the membrane that was stretched on the tube to form donor and acceptor compartment.

The assay could be affected from the membrane, media temperature, rotation speed, as well as the sampling time during *in vitro* assay.

Cellophane was used as the membrane for *in vitro* drug release assay of ophthalmic preparation. Media (STF) temperature was maintained at 37±0.5 °C to examine the polymer gelation thermo-responsive behavior of poloxamer 407. Rotation speed was set at 50 rpm to imitate the blinking response of the eyelid. When sampling was performed, the pressure exerted when sampling or injecting STF fluid onto the acceptor compartment should be constant to avoid the instability of the drug release yield.

From the obtained data, the chloramphenicol *in situ* gel rate of release for F1 = 0.038 (mg/l) h^{-1}, F2 = 0.056 (mg/l) h^{-1}, F3 = 0.064 (mg/l) h^{-1}, dan F4 = 0.027 (mg/l) h^{-1}. Based on chloramphenicol rate of release from *in situ* gel preparation, it can be concluded that, the faster the release rate of the drug, the retention time needed for the formula to be detected in the eye will also became faster, but the resulting bioavailability will be decreased due to the increased release rate that will make the retention of a drug in the eye become faster. F4 had the longest retention time when compared to F1, F2 and F3. According to Shasank [32], the longer the retention of a drug in the eye, the ocular bioavailability will be increased. So, dosing frequency will be reduced and patient adherence will improve. All formulas (F1–F4) had shown continuous release for 8 h.

The *in vitro* kinetic release of a drug was determined by calculating the correlation coefficient (r^2) for each model. If the r^2 value is closer to 1, the correlation between two relationships (Amount of drug release against time) will become more positive, and that was the chosen *in vitro* kinetic model.

From table 5 the *in vitro* kinetic release of chloramphenicol *in situ* gel formulas showed that F1 and F2 followed the zero-order model, F3 followed the higuchi model and F4 followed the Korsmeyer-Peppas model. The *n*-value in Korsmeyer-Peppas model greatly affected the release mechanism of a drug, which could be seen from the slope of the equation. From the *n*-value, F1, F2 and F4 followed the "*Supercase II*" mechanism, where the drug release followed the zero-order model and erosion mechanism. It described a condition where a formulation was eroded and the drug substance was detached from the formulation and came into contact with the media. F3 followed the "*Non fickian*" mechanism. It described a condition where a formulation followed the hybrid of diffusion and erosion mechanism. Diffusion mechanism describes that the drug will move through a barrier.

Factorial designs are used primarily for understanding if factors are important to the process. This can take the form of screening for a few important factors out of many possibilities or characterizing how known factors interact and individually affect the process. 2^2 factorial design was carried out to find the formula with the most desirable response value (cumulative amount of drug release). Response values were submitted into the software and it will show the most important factors on the intended response.

The half-normal plot on fig. 2 plots half-normal % probability value in y-axis and the absolute standardized effect x-axis. The half-normal % probability is not data-dependent; it is dependent only on the half-normal distribution and the number of items plotted (=*n*-1). The theoretical medians on the half-normal distribution represent an "ideal" typical ordered data set that would have been gained from a random drawing of (*n*-1) samples. Meanwhile, the absolute standardized effect gives the absolute value of the estimated effect of the main factors (poloxamer 407 or HPMC) and interactions (poloxamer 407 and HPMC) [35].

Usually, a normal plot is used to estimate the direction of the effect. But, the software already gave the direction of the effect, with the blue box having a negative effect and the orange box having a positive effect. HPMC had a contribution value of 0.66% and standardized effect of 2.28 (positive effect). Poloxamer 407 had a contribution value of 13.8% and standardized effect of-10.38 (negative effect). Combination of poloxamer 407 and HPMC had a contribution value of 82.01% and standardized effect of-25.41 (negative effect). Meanwhile, pure error between the estimated data and the observed data has its value of 3.65%.

From this analysis, only the increase of HPMC concentration would increase the amount of drug release rather than poloxamer 407 or combination between both excipients. It could be concluded from this study that the most important into the less important factors were AB (poloxamer 407 and HPMC), A (poloxamer 407) and B (HPMC). The viscosity of poloxamer 407 that was too high, will make the release of the drug become too difficult. Since 2-level full factorials are only used for screening design**,** the only available ANOVA regression model follows the 2 Factors of Interaction (2FI) model.

From table 6, the *p*-value from poloxamer 407 and the combination of poloxamer 407 and HPMC was lower than 0.05, which means the null hypothesis (H_0) was rejected and the alternative hypothesis (H_1) was accepted. From this experiment, there was a significant difference between poloxamer 407 and the combination of poloxamer 407 and HPMC from F1 to F4. While the *p*-value of HPMC was higher than 0.05, which means the null hypothesis (H_0) was accepted. From this experiment, there was no significant difference between HPMC from F1 to F4.

Fig. 3(a) showed the 3-dimensional relationship between cumulative amount of drug release, poloxamer 407 and HPMC in 2FI form. Fig. 3(b) showed the plotting of poloxamer 407 in x-axis, HPMC in y-axis and the cumulative amount of drug release in contour. Meanwhile, the mathematical relationship in form of 2FI regression (in terms of actual factors), which described the relationship between cumulative amount of drug release, poloxamer 407 and HPMC was explained by the following polynomial regression:

$$\begin{aligned}(\text{Cumulative Amount of Drug Release}(\%)) &= 11.324\,(\text{Poloxamer 407}) + 142.775(\text{HPMC}) \\ &\quad - 18.484(\text{Poloxamer 407.HPMC}) - 48.679\end{aligned}$$

Standard error analysis was used to examine the design accuracy and precision. If the standard error value is closer to 0, it indicates that the data have less deviations. From F1–F4, the standard error value is 0.577, which could be seen from the red dots. The less the standard error, the design's accuracy and precision will be better.

Fig. 5 showed that the higher poloxamer 407 concentration resulted in the decrease from the cumulative amount of drug release, but HPMC was increased. The decreasement in cumulative amount of drug release with a higher concentration of poloxamer 407 was significant and HPMC was not significant. It had been proven with the ANOVA analysis from the *p*-value on table 6.

Fig. 6 showed that the higher poloxamer 407 concentration, when maintained at HPMC 0.45%, will increase the cumulative amount of drug release. The higher poloxamer 407 concentration, when maintained at HPMC 1%, will decrease the cumulative amount of drug release. It can be concluded that the combination of poloxamer 407 when maintained at HPMC at 0.45% concentration, is the highest value cumulative amount of drug release.

The software would recommend a solution for the best composition of poloxamer 407 and HPMC based on cumulative amount of drug release by utilizing desirability value.

Desirability had the range from 0 to 1 with the contour color sequence from blue, green to red zone. The closer the desirability value to 1 (red zone), the software's ability to produce optimum formula is better. The closer the desirability value to 0 (blue zone), the software's ability to produce optimum formula is worsen. From table 7, the software gave three formulas (F2, F3 and F_{pred}) as solutions which yielded the best cumulative amount of drug release. The first solution gave a desirability value of 0.58 (F3), the second one with a desirability value of 0.54 (F_{pred}) and the last one with desirability value of 0.46 (F2).

From the contour plot shown in fig. 7(a) and the response surface plot shown in fig. 7(b), it can be concluded that the closer the desirability value to the blue region, the desirability value will be decreased, which means the resulting response progressively do not meet the satisfaction criteria. If the desirability value was closer to the green region, the desirability value will increase, which means the resulting response progressively meets the satisfaction criteria [26]. From this analysis, the best formula base composition for chloramphenicol *in situ* gel in terms of (%w/v) was F3 containing

poloxamer 407 5% w/v and HPMC 1% w/v based on cumulative amount of drug release.

CONCLUSION

The formula of chloramphenicol *in situ* gel could be optimized by using a two-level full factorial design based on the cumulative amount of drug release. Formula 3 (F3) with the best cumulative amount of drug release composed of poloxamer 407 5% w/v and HPMC 1% w/v which yielded 58.30% and desirability value of 0.58 and it was the most desirable response value.

ACKNOWLEDGMENT

The Authors are very thankful to Direktorat Riset dan Pengabdian pada Masyarakat dan Inovasi (DRPMI), Dean of Faculty of Pharmacy Universitas Padjadjaran for the support. And so to PT. Megasetia Agung Kimia for support donations in kind (poloxamer 407 and HPMC used for experiments).

FUNDING

This research received no external funding

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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