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Research Article

The Effectiveness of Chloramphenicol In-Situ Opthalmic Gel with Base Poloxamer 407 and HPMC Against *Staphylococcus Aureus* Atcc 29213 And *Pseudomonas Aeruginosa* Atcc 27853

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ABSTRACT

Introduction: In-situ gel is a simple liquid transparent polymer solution under storage conditions, but turns into a viscoelastic gel after entering the eye due to the phase transition properties of the polymer that increase the residence time in ocular organ and bioavailability, enabling the delivery of reproducible doses and improving patient compliance. The aim of the present study was to formulate and evaluate the antibacterial effectivity of chloramphenicol in-situ ophthalmic gel with base poloxamer 407 and HPMC base against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Material and Methods: The optimization of ophthalmic gel preparation by the factorial design method has been carried out in order to know the best formula of all the formulas employed with 0.5% chloramphenicol active substance, wherein each formula was obtained from high concentration and low concentration of each base. Results: The measurement of the antibacterial effectivity against Staphylococcus aureus ATCC 29213 and Pseudomonas aeruginosa ATCC 27853 by one-way ANOVA analysis showed that formula with base poloxamer 407 5% (F1) gave the best result. F1 has a dilute consistency, clear and stable during 28 days storage time when effectiveness test performed. Conclusions: Chloramphenicol in-situ gel with base poloxamer 407 and HPMC were effective against *Staphylococcus aureus* ATCC 29213 with intermediate to sensitive category, and *Pseudomonas aeruginosa* ATCC 27853 with sensitive category in accordance to the requirements of the Clinical and Laboratory Standards Institute (CLSI).

Keywords: chloramphenicol, HPMC, in-situ gel, poloxamer 407, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

INTRODUCTIONS

In-situ gel is a simple liquid transparent polymer solution under storage conditions, but turns into a viscoelastic gel after entering the eye due to the phase transition properties of the polymer. The advantage of this gel is to increase the residence time in ocular organ and bioavailability; enabling the delivery of reproducible doses and improving patient compliance[1]. The phase transition from solution to gel to in-situ gel occurs because the polymers incorporated in the system may undergo modifications caused by changes in temperature, pH or lacrimal fluid electrolyte composition[2]. Various polymer combinations have been successfully used for the desired formulation. A good ophthalmic in-situ gel should have a liquid gel transition temperature greater than room temperature and form a gel at pre-corneal temperature (35°C) and should not be store at cold temperatures before use, as it may cause eye irritation due to its cold nature[3]. In-situ ophthalmic gel usually used polymer base such as Sodium Alginate, Poloksamer 407, HPMC and others. The gel bases used are Poloxamer 407 and HPMC wherein a single base is highly effective in the formulation of ophthalmic gel preparations. Poloksamer 407 offers a new strategy for improving bioavailability and reducing side effects caused by systemic uptake of topically applied ophthalmic drugs even though HPMC has very low irritating power, has a compability with many acidic, alkaline substances and neutral, and chemically stable[4]. In designing an in-situ gel formula, a method is required to produce an optimal formulation, one of which is by factorial design.

Factorial design is used in experiments in which the effects of various factors or conditions of simultaneous selection of the effects of several factors and their interactions. The design is formed based on a number of levels of each factor to be observed, then experiments on all possible factor-level combinations. Furthermore, these factors are observed together to show whether there is influence or interaction effect between factors[5]. One of the factors in this study is the minimum inhibitory concentration. Minimum inhibitory concentration (MIC) is regarded as a standard for determining the

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Table 1: Formulation of ophthalmic preparations for poloxamer base 407 and HPMC.

Ingredients	Concentrations % (b/v)			
	F1	F2	F3	F4
Chloramphenicol	0.5	0.5	0.5	0.5
Poloxamer 407	5	10	-	-
HPMC	-	-	0,45	1
Propylenglycol	10	10	10	10
Propylparaben	0.01	0.01	0.01	0.01
Aquadestilata	add until 100 mL			

Table 2: Optimized base results without active ingredient.

Material	Concentration % (b/v)			
	F1	F2	F3	F4
Poloxamer 407	5	10	-	-
HPMC	-	-	0.45	1
Aquadestilata	add 100 mL			

susceptibility of an organism to antimicrobials and is therefore used to assess the performance of all other testing methods of contradiction. MICs are used in the diagnostic laboratory to confirm unusual resistance, providing definitive answers when boundary results are obtained by other testing methods. The range of antibiotic concentrations used to determine the MIC universally in the dilution step is doubled up and down from 1 milligram per liter as needed. MIC is defined as the lowest concentration of drug that will inhibit the growth of organisms seen after overnight incubation (this period is extended for organisms such as anaerobes, which require incubation for prolonged growth[6].

METHODS

Tools

The tools used in this research were stirrer, bacteria filter (Minisart®), petri dish, erlenmeyer 500ml (Pyrex®), filter paper, laminar air flow (Esco ™), spiritus, loop, oven (Heraeus®), test tube, analytical scales (Metler Tolledo®), autoclave (All American®), incubators (Memmert®), 10-100µl micropipette, perforator, Design Expert® (factorial design) software (version 8.0.0), SPSS Statistic software ® (ANOVA) (version 22), dropper and test tube rack.

Materials

The materials used in this research were 70% alcohol, aqua bidestilata sterile, *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), Fluid Thioglycollate Medium (Oxiod®), hydroxypropyl methylcellulose (HPMC), chloramphenicol (BioBasic®), Mcfarland solution (sulfuric acid and barium chloride), poloxamer-407 (Kolliphor®P 407), propylenglycol, propyl paraben (Nipasol) (Merck®), Mueller-Hinton Agar (Oxoid®), Tryptone Soya Broth (Oxiod®) and NaCl physiology.

Procedures

Preformulation of in situ Gel

The pre-formulated test of ophthalmic *in situ* gel preparations includes an examination of the active substance of chloramphenicol used in accordance with the Indonesian Pharmacopoeia V Edition, additives poloxamer 407, HPMC and others in accordance with the Handbook of Pharmaceutical Excipients.[7]

Optimization of In situ Base of Ophthalmic Gel

The data obtained from the formulation based on the 2 level factorial design method (optimum and verification result) were analyzed using Design Expert ® version 8 software with 95% confidence level and t test if there is a significant difference[5]. Optimization of base (Poloxamer 407 and HPMC) using factorial design. The result of base optimization was found concentration of poloxamer 407 was 5% and 10%, while HPMC concentration was 0.45% and 1%.

Ophthalmic Gel in Dosage Formulation Form

The basic optimization was done by using a 2-level factorial design software, then select the variable 22 on the column and change the replication to 3 times. The base factor used was poloxamer 407 and HPMC with a range of levels of each substance and the base variable will randomize the formulation. Ophthalmic gel *in situ* preparations were prepared according to the formula in Table 1. The formula was the result of basic optimization using factorial design. The preparation of the ophthalmic *in situ* gel was prepared by the procedure as follows: F1 of 5 grams of poloxamer 407 was weighed. F2 weighed as much as 10 grams poloxamer 407. Next F3 weighed as much as 0.45 gram HPMC. And the last F4 weighed as much as 1 gram HPMC. Poloxamer 407 dissolved using a cold aquadest and HPMC dissolved using a hot aquadest, and continued by mixing the two solutions according to the formula. Next 0.5 gram of chloramphenicol was weighed and then dissolved into 10 mL propyleneglycol until dissolved completely. Propylparaben (nipasol) used as a preservative, weighed as much as 0.01 grams and dissolved in sufficient heat aquadest. After that the chloramphenicol soluble with propyleneglycol was mixed with nipasol and stir back to homogeneous. Each solution in ad of 100 mL aquadest was then fed into 10 vials each containing 10 mL[8].

Evaluation of Antimicrobial Effectiveness of the Preparation

Mueller-Hinton Agar (MHA)

15.2 grams of MHA powder were weighed, then dissolved in 400ml aquabidest. The solution was heated there the solution showed clear (yellow). Then put in autoclave for sterilization for 45 minutes[9].

Standard Mc Farland Solution 0.5

Table 3: Standard category of inhibition zone diameter for *Staphylococcus aureus* bacteria.

Type of Antibiotic	Concentration Disc of Antibiotic	Inhibition Zone Diameter (mm)		
		Sensitive	Intermediate	Resistant
Chloramphenicol	30µg	≥18	13 to 17	≤12

Tabel 4: Result of inhibition zone bacterial antibacterial *Staphylococcus aureus* ATCC 29213 during 28 day storage (mm).

Formula	Storage Day-							
	0	1	3	5	7	14	21	28
1	19.3± 1.32	21.0± 0.50	20.6± 0.60	21.0± 0.50	20.0± 0.50	18.4± 0.52	17.3± 0.50	16.6± 0.52
2	20.0± 0.87	20.5± 0.53	20.6± 0.53	20.0± 0.87	19.4± 0.53	18.5± 0.73	17.6± 0.53	15.9± 0.60
3	18.7± 1.00	21.2± 0.67	21.0± 0.67	20.3± 0.71	20.2± 0.60	18.3± 0.60	17.3± 0.50	15.9± 0.93
4	19.7± 0.50	20.6± 0.53	20.4± 0.73	20.0± 0.70	19.5± 0.53	18.8± 1.48	17.4± 0.53	15.8± 0.67
Raw Chloramphenicol	20.3± 0.55	20.1± 0.33	20.3± 0.73	20.0± 0.71	19.7± 0.67	18.4± 0.53	18.4± 0.53	18.6± 0.50

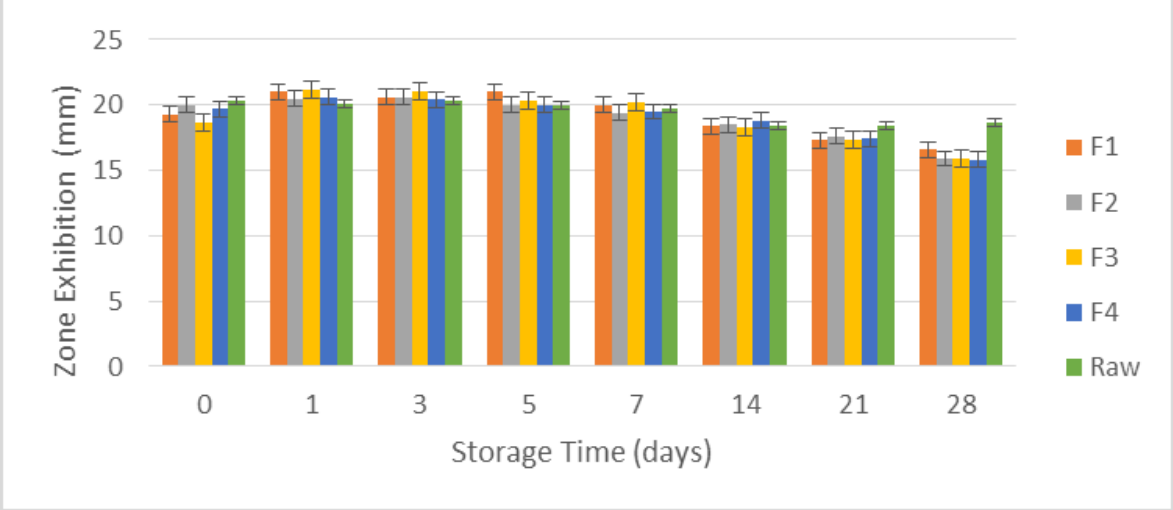

Figure 1: Graph of inhibition zone result of *Staphylococcus aureus* ATCC 29213 antibacterials during 28 days of store storage.

0.05 ml 0.048 M barium chloride (1.175% w / v) was weighed, 9.95 ml 0.18 M sulfuric acid (1% v / v) and then mixed. The mixture was shaked with a vortex mixer. Then, measured by a spectrophotometer in a wavelength of 625 nm. Observe the absorbance of standard solutions. Close the tube tightly and keep it at room temperature in the dark. This solution will be stable for at least 6 months. To use it, shake the tube several times (or use a vortex mixer) to resuspend the barium sulphate deposit. The best comparison between bacteria and Mc Farland is done using a background of a black and white horizontal line[10].

Sterility Test

The sterility test of an ophtalmic gel preparation was performed using Fluid Thioglycollate Medium (FTM) media and Tryptone Soya Broth (TSB) media. Aseptically, each test preparation was inoculated into a reaction tube containing FTM media and incubated at a temperature of 30-35^{0}C for not less than 14 days. The occurrence of turbidity in the test tube is observed daily. To the reaction tube containing the TSB medium, inoculation of the test preparation was then incubated at a temperature of 20-25°C for less than 14 days. The occurrence of turbidity in the test tube is observed daily[11].

Antimicrobial Effectiveness Test

The test was performed by diffusion method in order for the storage days at 0, 1, 3, 5, 7, 14, 21, and 28 days. The chloramphenicol base was used as a comparison (standard). The same treatment was made for the active substance of the sample. Planned dilution of sample solution and standard solution in the same dose variation. The standard solution and the developed formulation (test solution) brought into separate cups into sterile MHA were previously seeded with organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). The bottom surface of the petri dish was divided into five equal areas. A 20 µL suspension test for *Staphylococcus aureus* and *Pseudomonas aeruginosa* with equivalent turbidity level of 0.5 mF (0.5 x 108 cfu/mL) was poured into 20 mL agar medium (45°C). The media mixture with the bacterial suspension was homogenized and allowed it to solidify. Labeled each area depending on the variety of doses to be used. Aseptically, five reservoirs were prepared in a petri dish by using a perforator 9 mm. Into the hole on the test medium, the sample solution on the market and the diluted sample solution, were included with the variation of the test concentration using the micropipette. As a control, there were also positive controls in the form of media inoculated with suspension of test bacteria. As for the negative control used solid media without any treatment. All test and control media

Tabel 5: Result of inhibition zone bacterial antibacterial *Pseudomonas aeruginosa* ATCC 27853 during 28 day storage (mm).

Formula	Storage Day-							
	0	1	3	5	7	14	21	28
1	23.3± 0.50	24.7± 0.50	23.7± 0.50	24.0± 0.00	23.7± 0.50	22.7± 0.50	22.3± 0.50	21.7± 0.50
2	22.3± 0.50	22.7± 0.50	22.3± 0.50	22.7± 0.50	22.7± 0.50	21.9± 0.60	21.7± 0.50	21.3± 0.50
3	24.3± 0.50	22.3± 0.50	23.3± 0.50	22.3± 0.50	23.7± 0.50	22.0± 0.87	21.3± 0.50	21.0± 0.00
4	24.3± 0.50	23.9± 0.93	24.0± 0.00	23.7± 0.50	23.3± 0.50	21.3± 0.50	21.3± 0.50	21.0± 0.00
Raw Chloramphenicol	23.7± 0.50	24.3± 0.50	24.0± 0.00	23.3± 0.50	24.0± 0.00	23.7± 0.50	23.7± 0.50	23.3± 0.50

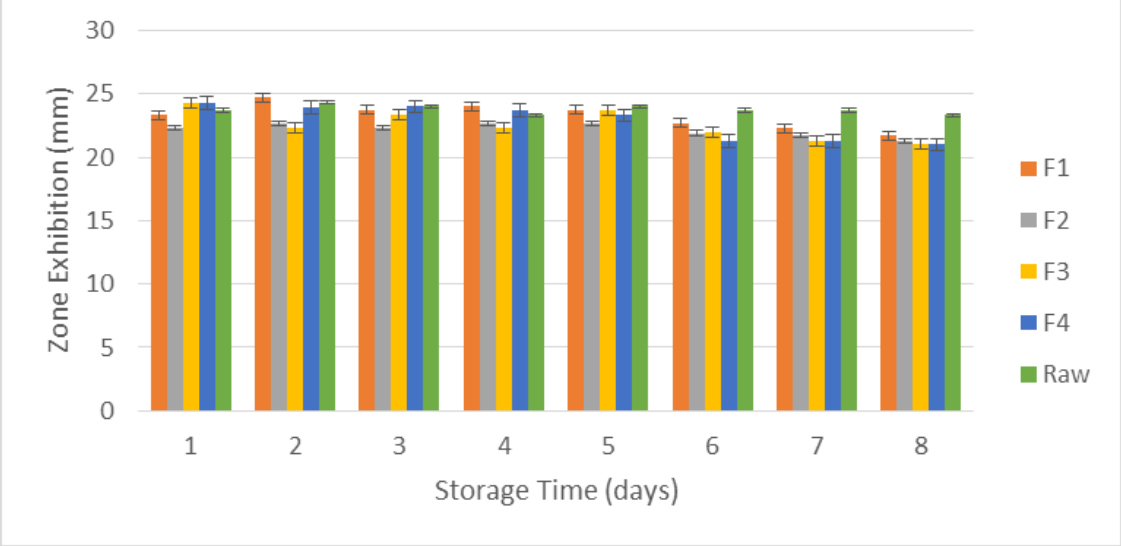

Figure 2: Graph of inhibition zone result of *Pseudomonas aeruginosa* ATCC 27853 antibacterials during 28 days of store storage

were incubated at 37°C for 18-20 hours. Observations of the effectiveness of antimicrobials were performed by measuring the diameter of the clear zone (lysis zone) occurring around the reservoir containing the antibiotic using a sliding range. Calculated antibiotic resistance zone formed using a transparent ruler[12].

RESULTS AND DISCUSSIONS

Preformulation Dosage of in situ Ophtalmic Gel

Preformulation of in-situ ophthalmic gel preparations carried out by chloramphenicol efficacy test on bacteria *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. Chloramphenicol efficacy test was performed to determine the potential resistance of raw chloramphenicol to be used in the formulation.

In situ Gel Dosage Formulations

The formulation of *in-situ* chloramphenicol gel preparation with the addition of poloxamer-407 and HPMC base produces clear preparations according to the requirements.

Design Dosage Form of In-situ Ophthalmic Gel

The design of the formula was based on the optimization result of the base combination formula using 2 factorial factorial design 2 levels with 95% confidence level, then the variable 2^2 was chosen in the column and converted into three repetitions. The incorporated base factor used is poloxamer 407 and HPMC with a range of levels of each substance adjusting the requirements of Pharmacope IV and Handbook of Pharmaceutical Excipients and the base variable will randomize the formula. After the design is done in accordance with the established procedures, then obtained the results of the basic optimization as listed in Table 2.

Optimization of In-Situ Ophthalmic Gel Formulations

The result of base optimization was obtained by twelve base formula. The gel bases used were Poloxamer 407 and HPMC wherein a single base is highly effective in the formulation of ophthalmic gel preparations. Poloxamer 407 offers a new strategy for improving bioavailability and reduces side effects caused by systemic uptake of topically applied ophthalmic drugs even though HPMC has very low irritation, has a high degree of acidic, and neutral, and chemically stable.[4] Based on the evaluation results, from the four base formulas, F1 was the formula chosen to be formulated with the addition of the base poloksamer 407 of 5 grams. F2 was a formula selected for formulation with the addition of a base poloksamer 407 of 10 grams. F3 was

Table 6: Result of sterility test of *in situ* ophthalmic gel.

Day	Formula							
	F1		F2		F3		F4	
	FTM	TSB	FTM	TSB	FTM	TSB	FTM	TSB
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-

Description :
(+) : bacterial / fungal growth
(-) : no bacterial / fungal growth

the formula chosen for formulation with addition of base that was HPMC equal to 0.45 gram and F4 was formula chosen to be formulated with addition of base that was HPMC equal to 1 gram. The concentration of chloramphenicol active substance used was 0.5% where the concentration has been tested for its effectiveness in the treatment of eye infections[13]. The result of a sterile gel ophthalmic dosage formulation containing chloramphenicol-produced active substances with aseptic and final sterilized using a bacterial filter.

Antibacterial Effectiveness Test of in situ Ophthalmic Gel

The antibacterial effectiveness test was performed on bacteria *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 aims to know the effectiveness of ophthalmic gel preparations that have been made. Staphylococcus aureus which is a gram-positive bacteria while Pseudomonas aeruginosa bacteria is a gram-negative bacteria. In this study selected Mueller Hinton Agar (MHA) because this media has been recommended by the FDA and WHO for antibacterial (sensitivity) tests especially aerobic bacteria and facultative anaerobic bacteria as food and clinical material. The media for this has also been proven to produce good and reproducible results. These media contain sulfonamides, trimethoprims, and low tetracycline inhibitors and provide satisfactory pathogenic growth[11]. Selection of MHA media in this study was also conducted by reason of the test is based on the principle of inhibiting zone calculation using diffusion method. Aseptically, the diffusion method is to make five reservoir molds in a petri dish by using a 9 mm perforator.

Then, the bacterial suspension was taken using one loop of bacterium Staphylococcus aureus and Pseudomonas aeruginosa each put into a 20ml reaction tube then compared with a standard solution of Mc Farland 0.5. The Mc Farland standard 0.5 solution was used as a reference to adjust the bacterial suspension turbidity so that the bacterial count would be within the range provided for standardized microbial testing and aims to determine the approximate cell concentrations in bacterial suspensions[10,14]. In this study using the diffusion method agar by incorporating the concentration of the appropriate antibiotic disc in the CLSI standard requirements which mixing the agar and make sure the media agar plate are dissolved homogeneously. In the measurement results the diameter of the antibiotic inhibitory zone can be divided into 3 categories based on Clinical and Laboratory Standards Institute (CLSI) standards.

Source: Clinical and Laboratory Standards Institute[15].

Table 3 was used as a reference to know the results of the stock evaluation including the drag zone diameter in sensitive, intermediate, or resistant category. Concentrations of antibiotic discs used as a reference for 30 µg, whereas in testing of inhalmate gel preparations in situ dilution that will cause the concentration of antibiotic discs used are smaller than 30 µg that is equal to 16.67 µg. This will affect the 3 categories of drag zone diameters that will be smaller when compared to the reference zone diameter zone shown in Table 3. The results obtained quantitative to prove that the preparations that have been made still provide effectiveness on the growth of bacteria, can be said to be sensitive to Staphylococcus aureus bacteria. The test was performed for 28 days of storage on days 0, 1, 3, 7, 14, 21, and 28 days by comparing the diameter of the inhibitory zone (lysis zone) of the test preparation with pure chloramphenicol. This is because chloramphenicol is a broad-spectrum antibiotic that can inhibit the activity of bacterial ribosomes, in addition *Staphylococcus aureus* bacteria are opportunistic more sensitive to antibiotic treatment. The results of the effectiveness test of each formula can be seen in Table 4.

Can be seen in the table, there was a decrease in inhibition zone (lysis zone) of bacteria ATCC 29213 *Staphylococcus aureus* during the storage period for 28

days. The inhibitory zone diameter of each formula along with the standard chloramphenicol resin zone diameter (pure chloramphenicol) is more evident in Fig. 4. Can be seen in the table, there was a decrease in inhibition zone (lysis zone) of ATCC 29213 Staphylococcus aureus bacteria during the storage period for 28 days. The inhibitory zone diameter of each formula along with the standard chloramphenicol resin zone diameter (pure chloramphenicol) is more evident in Fig. 1.

The results obtained quantitative to prove that the preparations that have been made still provide effectiveness on the growth of bacteria, can be said to be sensitive to bacteria *Pseudomonas aeruginosa*. This test was performed for 28 days of storage on days 0, 1, 3, 5, 7, 14, 21, and 28 days by comparing the diameter of the inhibitory zone (lysis zone) of the test preparation with pure chloramphenicol. This is because chloramphenicol is a broad-spectrum antibiotic that can inhibit the activity of bacterial ribosomes, besides *Pseudomonas aeruginosa* bacteria is a opportunistic pathogen, resulting in green fluorescent pigments and is not sensitive to any antibiotic preparations. The results of the effectiveness test of each formula can be seen in Table 5.

Can be seen in the table, there is a decrease in inhibition zone (zone lysis) bacteria *Pseudomonas aeruginosa* ATCC 27853 during storage period for 28 days. The inhibitory zone diameter of each formula along with the standard chloramphenicol resin zone diameter (pure chloramphenicol) is more evident in Fig. 2.

The results of the measurement of the effectiveness of antibacterial Staphylococcus aureus ATCC 29213 and Pseudomonas aeruginosa ATCC 27853 produced by the preparation contain chloramphenicol active substance in the first formula until the fourth formula gives sensitive results and has a drag zone diameter.

Sterility Test

The sterility test is performed on sterile form of *in situ* ophthalmic gel preparation which has been formulated. The results of sterility checks performed for fourteen days can be seen in Table 6.

From Table 6 it can be seen that in all formula of ophthalmic gel preparation does not occur the growth of bacteria or fungi. The purpose of the sterility test is to ensure that all products made do not contain microorganisms or are in fact contaminated[11]. This proves that the whole formula meets the sterility requirement, which is free from life-giving microbes. Thus, the aseptic formulation and autoclave sterilization method can be used to prepare an ophthalmic gel formula in accordance with the sterility requirements. However, it should be reassured that the absence of bacterial or fungal growth caused by the influence of other factors such as preservatives because preservatives can interfere with the results of sterility tests lead to false negative results. It is necessary to inactivate the preservative used at the time of sterility test of ophthalmic gel preparation.

Statistical Analysis

Data processing uses one-way ANOVA statistical method (One way anova) by utilizing the IBM SPSS software application. ANOVA analyzes the variability or diversity of data into two sources of variation, ie variations within group and inter-group variation (between). If the variations within and between are equal then the resulting average there is no difference, vice versa if the result of comparison of the two variants yields more than 1, then the average compared to show the difference.

In doing hypothesis, usually there are two kinds of mistakes, namely mistakes type one and type two errors. The error of type one or commonly expressed by the symbol α is a mistake made when rejecting the hypothesis that should be accepted. The value of α expresses significance in general health research using a significance level of 0.05. A significance level of 0.05 or 5% can be interpreted to occur 5 errors from a total of 100 errors. Simply put, the α level is defined as the probability of error we make when concluding that H0 is false, which turns out the H0 statement is true. Practically, the level of α is expressed in terms of chance or probability or p-value. If the resulting probability value is smaller than the predetermined α level value of 0.05 then the hypothesis must be rejected. This rejection is done because the error is smaller than the error parameter specified[16].

The SPSS statistical method generates ANOVA analysis. In ANOVA analysis, the initial hypothesis is determined that hypothesis 0 or H0 that poloxamer 407 or HPMC do not give effect (effectiveness) to drag zone diameter. While hypothesis 1 or H1 that HPMC give effect (effectiveness) to drag zone diameter. ANOVA analysis results show significantly less significance value than 0.05. This indicates that the results obtained significantly with H1 received ie the use of poloxamer 407 (F1 and F2) and HPMC (F3 and F4) both have an effect on the in situ zone diameter of *in situ* ophthalmic inhibition zone. The value of f (f-value) is one of the parameters used in deciding to reject or accept H0.

ANOVA results for the effectiveness of antibacterial *Staphylococcus aureus* ATCC 29213 showed *in-situ* ophthalmic gel Formula 1 (F1) had an F-value of 58.697, and a p-value of less than 0.05. In Formulation 2 (F2) has an F value of 54.785 and a p-value of less than 0.05. Formulation 3 (F3) has a T grade of 64.417 and the python p is less than 0.05. Formulation 4 (F4) has a F-value of 42.075 and a p-value of less than 0.05 when a pure chloramphenicol formulation (F4) has a F-value of 20.283 and a p-value of less than 0.05. Then the formulation all reject H0 if Fcount > Ftable, accept in other case. For this data, Fcount is greater than Ftable so H0 is rejected. This means that the use of all formulations has an effect on the inhalation zone inhales zone inhibition zone. The formulations all have an effect on the inhaler gel density inhibition zone inhibition zone diameter.

ANOVA results for the effectiveness of antibacterial *Pseudomonas aeruginosa* ATCC 27853 showed *in-situ* ophthalmic gel preparation Formula 1 (F1) has a F-value of 38.875 and a p-value of less than 0.05. In Formulation 2 (F2) has an F-value of 8.872 and a p-value of less than 0.05. Formulation 3 (F3) has a F-value of 42.603 and a p-value of less than 0.05. Formulation 4 (F4) has a F-value

of 65.895 and a p-value of less than 0.05 when a pure chloramphenicol formulation (F4) has an F-value of 5.714 and a p-value of less than 0.05. Then formulate all rejects H0 if Fcount > Ftable, accept in other case, for this data, Fcount is greater than Ftable so H0 is rejected. This means that the use of all formulations has an effect on the inhalation zone inhales zone inhibition zone. The formulations all have an effect on the inhaled gel inhaled dosage zone inhibition zone diameter. All formulations have an effect on the inhalant ophthalmic zone inhibition zone inhibition zone.

CONCLUSION

The formulation of in-situ chloramphenicol gel preparation using poloxamer-407 base and HPMC produces preparations according to the requirements set. Formulation of ophthalmic preparations of in-situ chloramphenicol gel with base Poloxamer 407 5% ie Formula 1 (F1) gave the best antimicrobial effectivity against *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 for 28 days of storage time.

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