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Research Article

Formulation of Chloramphenicol *In situ* Ophthalmic Gels Using Different Matrix Combinations

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ABSTRACT

In situ ophthalmic gel is solution which form a gel when it is placed onto the eyes. It is intended to have a longer contact time in order to improve the response of therapy. In this experiment, *In situ* ophthalmic gels were formulated by using Chloramphenicol 0.5% and matrix combination of either 0.4% Hydroxy Propyl Cellulose (HPC) or 0.2% Hydroxy Propyl Methyl Cellulose (HPMC) with Sodium Alginate 0.4, 0.5 and 0.6%. All formulas were sterilized by autoclave at 115^0C for 30 minutes. Physical evaluation including organoleptic, pH, viscosity, and drug content showed that all formulas fulfilled the ophthalmic gel requirements according to USP and Indonesian Pharmacopoiea. From rheological study, it can be concluded that the gels had pseudoplastic flow. Either matrix combination of HPC or HPMC with sodium alginate were in solution form during storage and formed a gel once contacted with Simulated Tear Fluids (STF) at 37ºC, which reflected eyes condition. Chloramphenicol release from all *in situ* gels formulations showed sustained release profiles, and 80% release of drugs were retarded to four hours. Antibiotic potency tests of ophthalmic *in situ* gels showed that formulation with HPC and sodium alginate matrix (F1, F2 and F3) had potency of 99.22, 98.45, and 96.97% against *Staphylococcus aureus* as well as 96.46, 96.68, and 96.49% against *Pseudomonas aeruginosa*, while that with HPMC and sodium alginate matrix (F4, F5 and F6) had potency of 99.87, 98.45 and 95.58 against *Staphylococcus aureus* as well as 97.66, 95.05 and 94.42% against *Pseudomonas aeruginosa.* It can be concluded that in situ gel system with either HPC or HPMC in combination with sodium alginate provide sustained release of chloramphenicol. Formulation process, including sterilization did not affect antibiotic potency since it remained in the range of potency value requirements.

Keywords: *In situ* ophthalmic gel, Chloramphenicol, HPC, HPMC, Sodium Alginate

INTRODUCTION

Medical dosage form which frequently used for eye infection treatment are eye drops. Even so, batch of eye drops usually have a slow respond time due to tears dilution effect which are able to quickly eliminate the medical solution on the cornea[1]. Eye drop preparation typically has short contact time with eyes which causes low therapeutic response. For that reason, several approaches are then developed in order to formulate dosage form with better and robust release profile once the solution were contacted onto eyes[2,3]. *In situ* gel dosage form is a solution that experienced reversible transitional phase (sol-gel-sol) caused by changes within the polymer due to formation process of complex structure as its response towards the environment[4]. It is a low viscosity solution; which has reversible transition phase (sol-gel-sol) as its response to physiological enviroment[4]. *In situ* ophthalmic gel has been developed to prolong contact time with eyes[5]. Generally, polymer is used to maintain drug release and prolong its time period. One type of polymer that is used as gelling agent is cellulose derivatives. Hydroxyl propylcellulose (HPC) and hydroxyl propyl methylcellulose (HPMC) are the most preferable of that type[5]. For *In situ* gel dosage form, Sodium alginate which is able to form a gel with calcium, one of the ions within the tears, can also be used[6,7]. Sodium alginate can also be used as matrix; it has structure that is able to form complex structure with calcium ion in tear fluid, resulting in gel formation[8,9]. Pathogen microorganisms, especially bacteria, are able to grow inside the eyes causing infection. The bacteria often to cause the infection are *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*. Chloramphenicol is an antibiotic with a wide spectrum that is able to deal with severe conjunctivitis in the eye, which was caused by the microorganism[10,11]. It is widely used to treat eyes conjunctivitis. Eye drop preparations are commonly sterilized in high temperature. However, antibiotics activity decreases if it has undergone hydrolysis, chemically (acid), physically (heating), and enzymatically. As a standard to overcome doubts about the possible loss of antibiotic activity (potency) towards its microbial resistance effect, it is necessary to do potential test of the drug after it is produced into pharmaceutical dosage forms. First study on formulation using only cellulose derivate as polymer matrix showed that in order to give prolonged drug release, high polymer level should be applied which in the end give high viscosity of gel, resulting poor flow property during application. The use of sodium alginate in

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Table 1: Chloramphenicol Ophthalmic *In Situ* Gel formulas.

S. No	Ingredients	Formula					
		F1	F2	F3	F4	F5	F6
1.	Chloramphenicol (%)	0.5	0.5	0.5	0,5	0,5	0,5
2.	HPC (%)	0.4	0.4	0.4	-	-	-
3.	HPMC (%)	-	-	-	0,2	0,2	0,2
4.	Sodium Alginate (%)	0.4	0.5	0.6	0,4	0,5	0,6
5.	Benzalkonium Chloride (%)	0,01	0,01	0,01	0,01	0,01	0,01
6.	Propylene glycol (%)	5	5	5	5	5	5
7.	HCl 0,1 N	ad pH 7,4	ad pH 7,4	ad pH 7,4	ad pH 7,2	ad pH 7,2	ad pH 7,2
8.	Aquabidest	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml

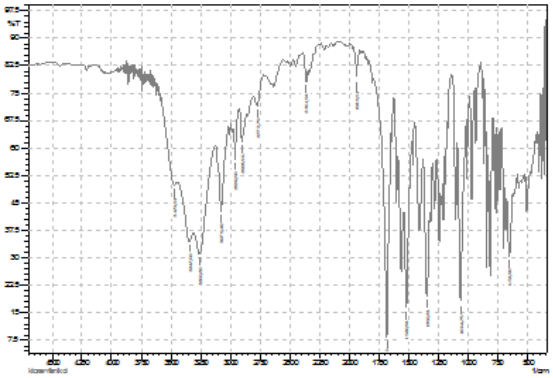


Figure 1: IR Spectra of Chloramphenicol (API).

combination with cellulose derivate theoretically could increase gel formation in order to improve time contact with eye mucous membrane. The preparation stayed as dilute solution during storage. The viscous gel of sodium alginate were formed once the solution contacted with Ca^{++} ions from tear fluids, resulting good matrix for prolonged drug release. The study on effect of polymer type and level on chloramphenicol release as *in situ* gels has not been published yet. Therefore, this research was aimed to study the effect of different matrix combination on release of chloramphenicol as *in situ* gels. The effects of formulation process on physicochemical performance including organoleptic evaluation, pH, viscosity, flow properties, drug content, and sterilization effect on antibiotic potency were also been studied.

MATERIALS AND METHODS

Materials

Chloramphenicol base (Bratachem), hydroxy propyl cellulose (HPC, Nippon Soda), hydroxy propyl methyl cellulose (HPMC, Bratachem), sodium alginate (Bratachem), sodium chloride (Bratachem), benzalkonium chloride (Merck), propylene glycol (Bratachem), hydrochloric acid (Merck), aquabidest, *Fluid Thioglycollate Medium* (FTM; Oxoid), *Tryptic Soy Broth* (TSB; Oxoid), sodium bicarbonate (Merck), calcium chloride dihydrate (Merck), potassium bromide (Merck), alcohol 70% (Brataco), pure isolate bacteria *Pseudomonas aeruginosa ATCC* 9027 and *Staphylococcus aureus ATCC* 25923 were used as received.

Methods

Chloramphenicol ophthalmic in situ gel formulation

The concentration of active pharmaceutical ingredient (API) chloramphenicol being used was 0,5% based on the chloramphenicol concentration in eye drops[12]. HPC concentration were 0.4%, while HPMC were 0.2% based on feasibility to form gels. Sodium alginate concentration were varied from 0.4 – 0.6%. Formulation of chloramphenicol *in situ* gels using different matrix combination shown in Table 1. HPC, HPMC and Sodium Alginate were dispersed in aquabidest separately and left overnight, until gel base were formed. Chloramphenicol was dissolved in propylene glycol. HPC or HPMC and sodium alginate gel bases were added into chloramphenicol solution and then added with benzalkonium chloride solution. The mixture were stirred vigorously by magnetic stirrer (Yellow-MAG HS7®), and sterilized using autoclave (Gea®), at 115ºC for 30 minutes.

Physicochemical examination

Compatibility study was determined by IR spectrofotometry (IR-Prestige Shimadzu®). It was conducted in order to determine the compatibility of the chloramphenicol with HPC, HPMC and sodium alginate used as excipients in the formulation. Physicochemical examination on *in situ* gels were included organoleptic evaluation, pH (Mettler- Toledo®), viscosity (TRADE Raypa®) and drug content, which calculated by UV spectrofotometry (Specord 200-Analytic Jena®).

Flow Properties Characterization

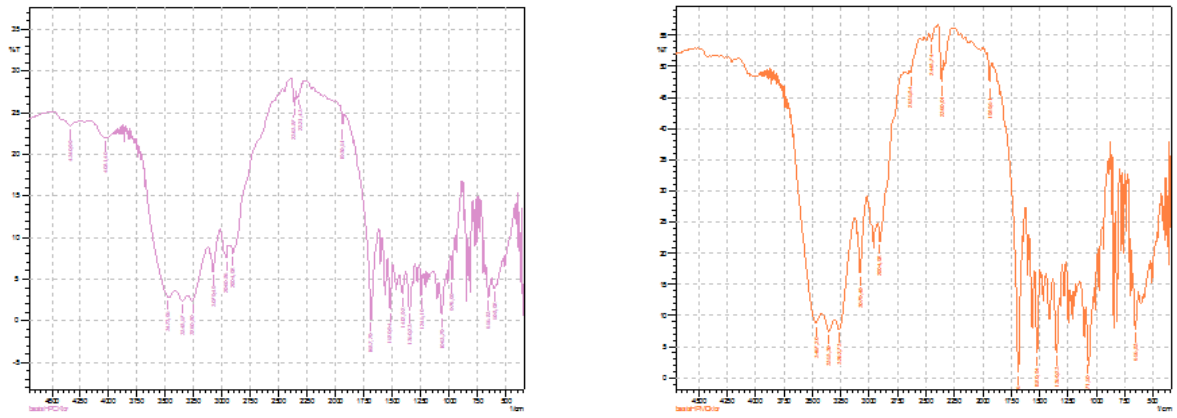
Evaluation of the Ophthalmic *In situ* Gel rheological properties were performed based on methods explained by Varshosaz and Mohanambal[8,9]. Rheological test was conducted by measuring viscosity of the formulation at different speed from 20, 30, 50, 60 to 100 rpm. The viscosity data were plotted against the shear rate and afterwards the flow characteristic can be determined.

pH and Temperature of Gelation determination

The *in situ* gel samples were heated starting from 25^{0}C, the pH and viscosity were measured. Simulated Tear Fluids (STF) having 37^{0}C temperature were added into the sample (40:7 ratio), and stirred at 50 rpm. The pH and temperatures of gelation were assumed as the sample solution formed a gel like substances after the addition of STF.

Drug release study

200 mL of eye STF were used as dissolution media. 2 mL of the *in situ* gel were introduced into the media and were stirred at 20 rpm. The samples were taken after 5; 10; 20;



(a) (b)

Figure 2: IR Spectrum of Chloramphenicol Mixed with (a) HPC+Sodium Alginat (b) HPMC+Sodium Alginat as polymer matrix for *in situ* gel.

Table 2: Absorbancy of functional groups in Chloramphenicol (API) before and after mixed with excipients.

Fungtional groups	Absorbance number (cm^{-1})	Chloramphenicol	Chloramphenicol +HPC+Sodium Alginate	Chloramphenicol+ HPMC+Sodium alginate
N-H	3000-3700	3474,91	3473,96	3467.20
O-H	3000-3700	3260,80	3260,80	3265.20
aromatic C-H	3000-3100	3079,49	3079,49	3079.49
C-H alkane	3000-2800	2903,96	2904,92	2904.92
aromatic C=C	1430-1600	1520,94	1520,94	1520.94
C-N	1180-1360	1350,23	1350,23	1350.23

Table 3: Physical Evaluation on Chloramphenicol Ophthalmic *In Situ* Gels.

Formula	Physical characteristics			Conclusion
	Organoleptic	pH	Viscosities (mPas)	
F1	Low viscosity solution, transparent, clear, odorless	7.1	46	*
F2	Low viscosity solution, transparent, clear, odorless	7,2	53	*
F3	Low viscosity solution, transparent, clear, odorless	7,1	59	*
F4	Low viscosity solution, transparent, clear, odorless	7.2	42	*
F5	Low viscosity solution, transparent, clear, odorless	7.2	58	*
F6	Low viscosity solution, transparent, clear, odorless	7.2	63	*
Requirements				
Organoleptic		Transparent, clear, odorless, free from coarse particles[13]		
pH		Ranges from 3,5 to 8,5[13]		
Viscosities		Ranges from 5 to 100 mPas[3]		

F1: Gel containing combination of 0.4 % HPC and 0.4% Sodium Alginate
F2: Gel containing combination of 0.4 % HPC and 0.5% Sodium Alginate
F3: Gel containing combination of 0.4 % HPC and 0.6% Sodium Alginate
F4: Gel containing combination of 0.2 % HPMC and 0.4% Sodium Alginate
F5: Gel containing combination of 0.2 % HPMC and 0.5% Sodium Alginate
F6: Gel containing combination of 0.2 % HPMC and 0.6% Sodium Alginate
(*): All of the parameters of the test have passed the requirement standards

30; 45; 60; 75; 90;120; 180; 240; 300; 360; 420; and 480 minutes. The drug content in samples were measured UV spectrophotometrically at 278 nm.

Antibacterial potency of chloramphenicol

Table 4: Chloramphenicol content on ophthalmic *in situ* gels.

Formula	Drug content (%)	Conclusion
F1	105.76	*
F2	105.82	*
F3		*
F4	105.08	*
F5	105.20	*
F6	105.95	*
Requirements		
Drug content	consist of no less that 90% and no more than 130% of Chloramphenicol[12]	

Table 5: pH and Temperature of Gelation of Chloramphenicol *In Situ* Ophthalmic *Gel*.

Formula	Temperature at 25°C		Temperature at 37°C (after STF was added)	
	Viscosities (mPas) at 50 rpm	pH	Viscosities (mPas) at 50 rpm	pH
F1	46	7,1	49	8,2
F2	53	7,2	59	8,4
F3	59	7,1	68	8,3
F4	42	7.2	44	8.2
F5	58	7.2	63	8.4
F6	62	7.2	69	8.2

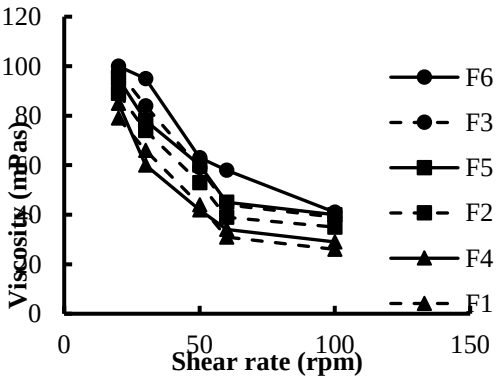


Figure 3: Rheogram of Ophthalmic Chloramphenicol *in situ* gel using HPMC (full line) and HPC (dash line) in combination with sodium alginate as polimer matrix.

The antibacterial potency tests on *in situ* gels were conducted by diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and reported as antibacterial potency of chloramphenicol after formulation. By using micropipette (50 µL), samples consisted of *in situ* gels and chloramphenicol standards solutions at three different concentrations were introduced into the reservoirs. The samples were then incubated on TZN4H Autonics®, at ± 37°C for 18 to 24 hours.

RESULTS AND DISCUSSION

Compatibility Study

Compatibility study between Chloramphenicol with HPC, HPMC and sodium alginate as polymer matrix were performed by comparing IR Spectrophotometry spectra between chloramphenicol as single drug substance with either chloramphenicol mixed with HPC and sodium alginate or mixed with HPMC and sodium alginate. The results showed that all excipients were compatible with Chloramphenicol as API, since the absorbance of functional groups before and after mixing remained relatively unchanged (Table 2, Fig. 1-2).

Physicochemical examination

The physical evaluation of chloramphenicol *in situ* ophthalmic gels were conducted including organoleptic examination, pH, and viscosity. The results can be seen in the Table 3. It can be concluded that all formulated ophthalmic *in situ* gels either using HPC or HPMC as gelling agent formed a clear, transparent low viscosity solution which were odorless. The pH range of all formula was between 7.1 – 7.2 which mean it fulfilled pH requirement for ophthalmic gel to which from 3.5 to 8.5. The viscosity values of all formula also fulfilled the requirement (in the range of 5 to 100 mPas). The increase in Sodium alginate concentration increased the value of viscosities, due to more polymer matrix in gels.

Drug content in Chloramphenicol ophthalmic in situ gels

Evaluation on drug content of ophthalmic *in situ* gels were conducted by diluting the samples in water. Chloramphenicol contents in the samples were measured by using UV spectrophotometry. Drug content of all formula fulfilled the USP requirement (in the range between 90 – 130%) as shown in Table 4. High content of drugs was due to water evaporation during sterilization process.

Flow Properties Characteristic

Rheological Test

Rheological test were conducted to determine the flow properties by observing the effect of shear rate on viscosity. The tests were performed at 20, 30, 50, 60 and 100 rpm. The rheogram showed that the flow property of the ophthalmic *in situ* gels were *pseudo plastic* (Fig. 3). It is a non-newton flow where the viscosity will decrease as the shear rate increases. The properties of p*seudo plastic* flow give the ease of ophthalmic gels to flow and coming out of the primary package so that it will be easier for application.

pH and Temperature of Gelation

Examination on pH and temperature of gelation were conducted to confirm gel formation after application of *in situ* gel (inside the eyes). The examinations were conducted at 25^0C which was the temperature at the time of storage and at 37^0C after addition of STF solution which reflected the condition similar to the eyes'. It was shown that there was an increase in viscosities value and pH of the formulas as it reaches 37^0C with *Simulated Tears Fluid* (STF) addition (Table 5). In order to give prolonged release of active drug, the ophthalmic *in situ* gel have to be able to form more viscous gel once it contacted with ion Ca^{2+} from tear fluids. In this experiment, the gel formation was due to the existence of sodium alginate, a substance which is able to form a gel when contacted with Ca^{2+} ions. Sodium alginate is a mixture between polyuronic acid, consisting of D-manuronnic acid and L-guluronic acid

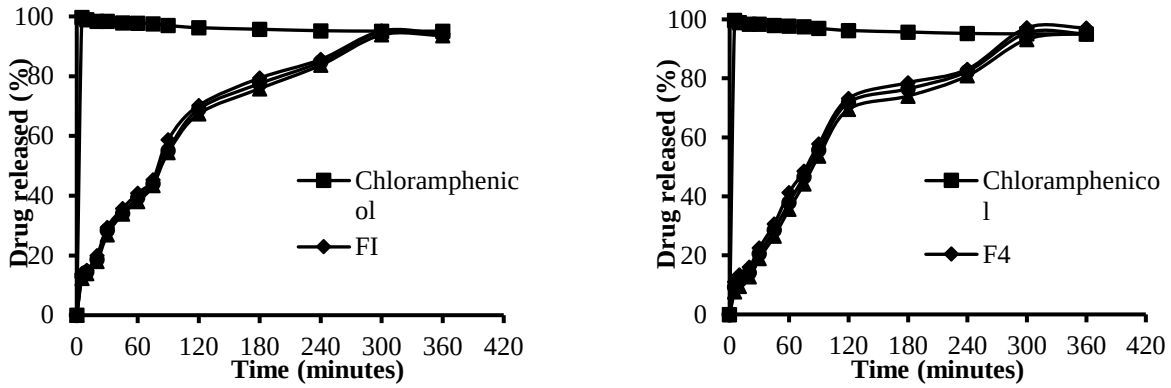


Figure 4: Release of Chloramphenicol as Ophthalmic *In Situ* Gel using matrix combination of HPC – Na Alginate (a) and HPMC – Na Alginate (b).

Table 6: Inhibition Zone for Antibiotics Potency determination of Chloramphenicol Ophthalmic *In Situ* Gel against *Staphylococcus aureus.*

Formula	Inhibition zone average of *in situ* gel sample (cm)			Inhibition zone average of chloramphenicol standard (cm)			Potency (%)
	S_T	S_M	S_R	B_T	B_M	B_R	
F1	2.245	1.810	1.633	2.210	1.845	1.643	99.22
F2	2.129	1.714	1.614	2.117	1.733	1.624	98.45
F3	2.026	1.712	1.493	2.108	1.645	1.516	96.97
F4	2.305	1.730	1.620	2.320	1.710	1.620	99.87
F5	2.298	1.890	1.593	2.304	1.821	1.642	98.45
F6	2.199	1.736	1.581	2.162	1.728	1.581	95.58

Table 7: Inhibition Zone for Antibiotics Potency determination of Chloramphenicol Ophthalmic *In Situ* Gel against *Pseudomonas aeruginosa.*

Formula	Inhibition zone average of *in situ* gel sample (cm)			Inhibition zone average of chloramphenicol standard (cm)			Potency (%)
	S_T	S_M	S_R	B_T	B_M	B_R	
F1	1.835	1.643	1.571	1.850	1.655	1.533	96.46
F2	1.842	1.623	1.531	1.867	1.632	1.521	96.68
F3	1.818	1.612	1.501	1.821	1.617	1.517	96.49
F4	1.964	1.719	1.511	1.946	1.706	1.520	97.66
F5	1.878	1.714	1.596	1.867	1.709	1.581	*95.08*
F6	1.798	1.710	1.573	1.780	1.665	1.570	*94.42*

F1: Gel containing combination of 0.4 % HPC and 0.4% Sodium Alginate
F2: Gel containing combination of 0.4 % HPC and 0.5% Sodium Alginate
F3: Gel containing combination of 0.4 % HPC and 0.6% Sodium Alginate
F4: Gel containing combination of 0.2 % HPMC and 0.4% Sodium Alginate
F5: Gel containing combination of 0.2 % HPMC and 0.5% Sodium Alginate
F6: Gel containing combination of 0.2 % HPMC and 0.6% Sodium Alginate
ST : *In situ* gel at high dosage
SM : *In situ* gel at medium dosage
SR : *In situ* gel at low dosage
BT : Chloramphenicol at high dosage
BM : Chloramphenicol at medium dosage
BR : Chloramphenicol at low dosage

residues. Alginate is able to form a gel because of the divalent cations like Ca^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}, where cross-bond occurred as a result of the chelate complex between the divalent ions and carboxylate anions from the G-G block. The gel is formed through a chemical reaction where calcium replaces sodium within the alginate[7]. The highest increase of viscosity value were achieved by the F3 and F6, due to the highest concentration of sodium alginate (0,6%). Thus it can be concluded that the higher sodium alginate concentration in the formula, the more gel formation occurred resulting more viscous gels. In terms of increase in pH value, the increased of pH up to 8.2 to 8.4 after addition of STF were still in the range of the

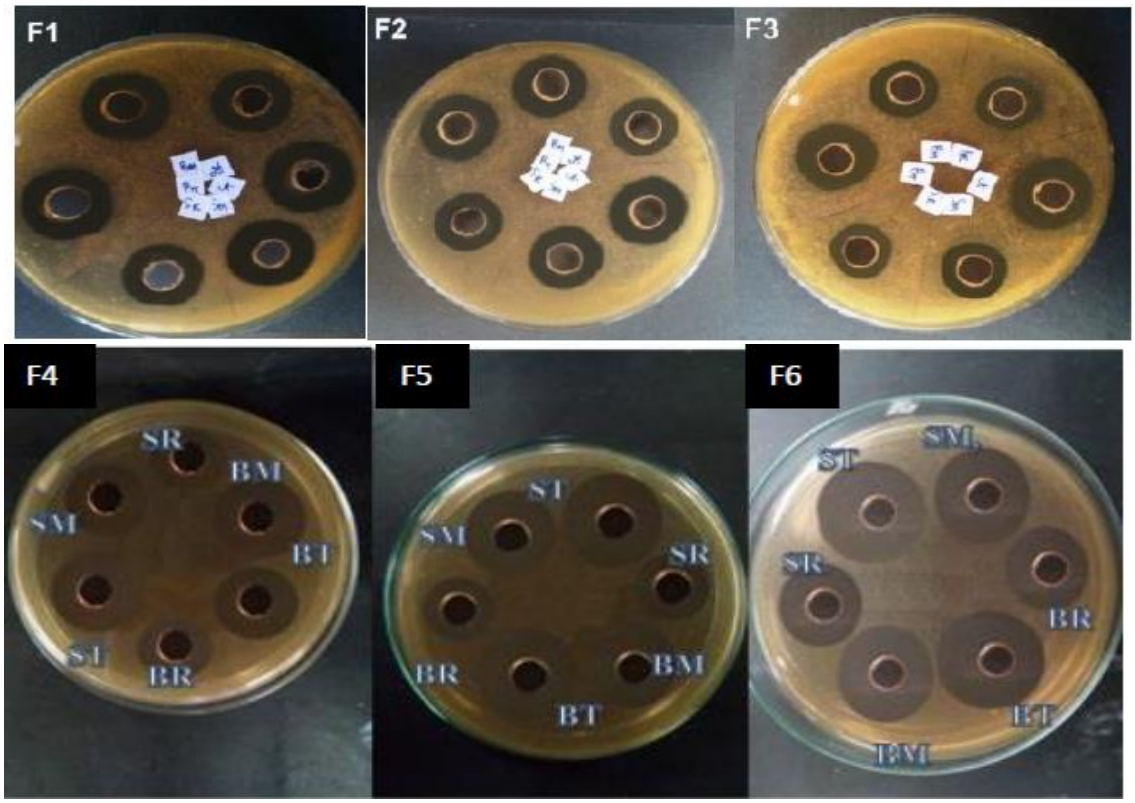


Figure 5: Antibiotics Potency test of Chloramphenicol as Ophthalmic *In Situ* Gel against *Staphylococcus aureus.*

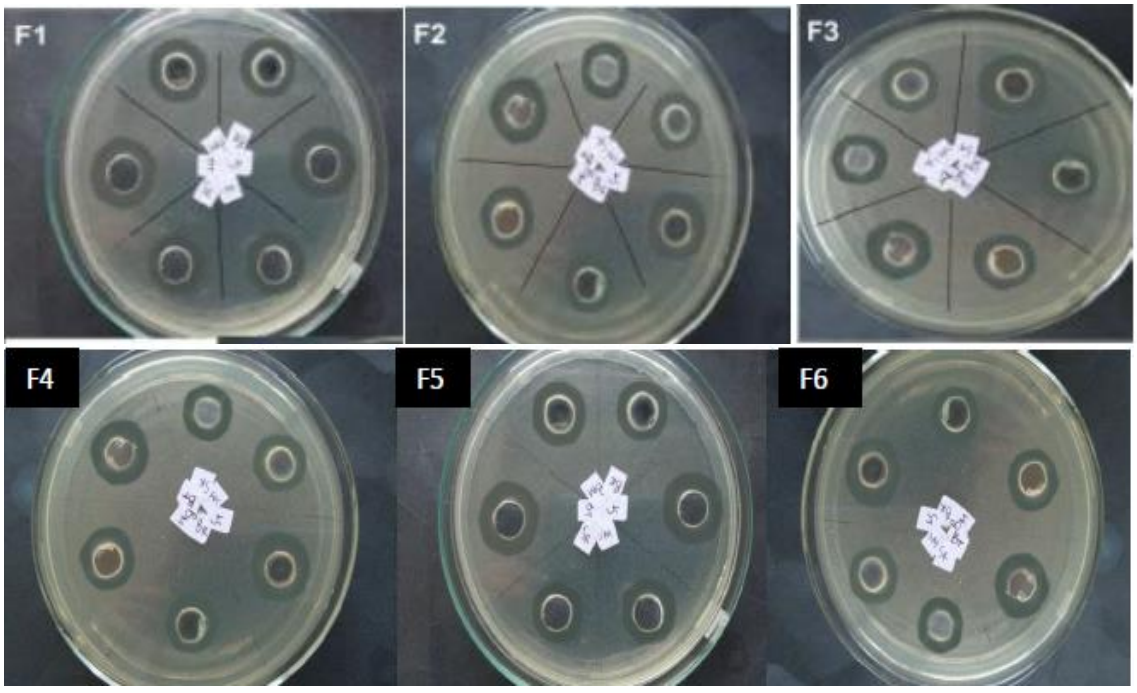


Figure 6: Antibiotics Potency Test of Chloramphenicol as Ophthalmic *In Situ* Gel against *Pseudomonas aeruginosa.*

standard pH value of the eye preparation in Farmakope Indonesia to which between 3.5 to 8.5[13].

Drug release study

This release study were conducted using the *Simulated Tears Fluid* (STF) medium since the environment condition has to be adjusted into the actual condition of the eye. The release profile of chloramphenicol from all formulation were compared with that of chloramphenicol as single substance. Compared to release profile as single substance, the release of chloramphenicol from *in situ* gel preparation were drastically sustained up into 4 hours to give 80% of drug either using HPC (Fig. 4a) or HPMC (Fig. 4b) in combination with sodium alginate as matrix. Sustained release of chloramphenicol from *in situ* gel was due to matrix formation following viscous gel formation after contact with STF. Addition of sodium alginate was

used to promote gelation of the matrix. Release of chloramphenicol from *in situ* gel of HPC and HPMC without combination with sodium alginate were poorly sustained, due to less viscous gel formation after STF addition (data was not shown). Statistical data analysis by ANAVA and Tukey showed that there is a real difference between drug release from *in situ* gel and pure chloramphenicol solution (single substance). There are no significant differences between all formulation (F1, F2, F3, F4, F5 and F6). It can be concluded that there is no significant effect either between type of matrix; HPC (in F1, F2 and F3) and HPMC (in F4, F5 and F6) and concentration of Sodium alginate on the release of chloramphenicol from *in situ* gels.

Antibiotic potency of Chloramphenicol as Ophthalmic In Situ Gels

The potency tests were conducted by diffusion method so that it could utilize perforation technique with three different dosages as requirement for potency examination. The antibiotic potency were determined based on the inhibition zone of samples including *in situ* gels containing chloramphenicol and chloramphenicol standard against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The result of diameter measurement of the inhibition zone shown in Table 6 and 7, as well as Figure 5 and 6. From the result in Table 6 and 7, it can be observed that the *in situ* ophthalmic gels potency were between 94.42%, to 99.87%, which mostly come to the ranges value for the antibiotics potency requirements shown on Indonesian Pharmacopeia (95% to 105%). This condition also signifies that neither level of polymer matrix nor type of polymer in *in situ* ophthalmic gels affected antibiotics potency. Accordingly, it can be observed that *Pseudomonas aeruginosa* was more sensitive than *Staphylococcus aureus* following larger zone inhibition.

CONCLUSION

Formulation of chloramphenicol as ophthalmic *in situ* gel dosage with matrix combination of HPC and sodium alginate or HMPC and sodium alginate was potential matrix system to give sustained release of chloramphenicol as treatment for eye's infection, since the release of 80% drug was retarded up to 240 minutes (4 hours). Physical mixing between chloramphenicol with polymer matrix combination of HPC and sodium alginate or HPMC and sodium alginate are compatible since there was not any new peak observed in IR spectrum compared to that of chloramphenicol as single substance. Rheology of in situ gels of all formulation were psedoplastic which form a dilute gel during storage and give the ease of gel for application, but once contacted with eye condition, it formed viscous gels which give the ability for active drug retardation. There was no effect of formulation either level of polymer matrix or type of polymer on antibiotic potency of chloramphenicol as *in situ* gels.

REFERENCES

1. Nagesh, C., Patil, M., Chandrashekhara S., Sutar R., 2012. A Novel In Situ Gel for Sustained Ophthalmic Delivery of Ciprofloxacin Hydrocholride and Dexamethasone Design and Characterization. *Der Pharmacia Lettre*. 4(3): 821-827.
2. Thermes F., Rozier A., Plazonnet B., 1992, Bioadhesion: the effect of polyacrylic acid on the ocular bioavailability of timolol. *Int. J. Pharm.*, 81: 59-65.
3. Patel H A., Patel J K., Patel K N., Patel R L. 2010. *Opthalmic Drugs Deivery System-A Review.* Der Pharmacia Lettre. 2 (4), India: Nootan Pharmacy College.
4. Mali M N and Ashok A H, 2010, *In Situ Gel- Forming Systems for Sustained Ocular Drug Delivery*, European Industrial Pharmacy. 17- 20.
5. Lachman L, Lieberman H A, 1994. *Teori dan Praktek Farmasi Industri, Edisi III.* Jakarta: Penerbit Universitas Indonesia, UI – Press. 643-645.
6. Wandrey C., 2005, *Polielectrolytes and Biopolymers*, Polytechnique Federale De Lausanne: Materials Science and Engineering. 37.
7. Inukai, M., Y. Masakatsu. 1999. Effect of Charge Density on Drug PermeabilityThrough Alginate Gel Membranes, *Chem. Pharm. Bul*., 47(8) 1059 – 1063.
8. Varshosaz J., Tabbakhian M., and Salmani Z., 2008, Designing of a Thermosensitive Chitosan/ Poloxamer In Situ Gel for Ocular Delivery of Ciprofloxacin, *The Open Drug Delivery Journal.* 2: 61- 70.
9. Mohanambal, E., Arun K and Abdul Hasan S. A., 2010, Formulation and Evaluation of pH-triggered in situ Gelling System of Levofloxacin, *India Journal of Pharmaceutical Education and Research*, 45(1): 58-64.
10. Siswandono and Soekardjo B., 2000, *Kimia Medisinal*, Edisi Kedua, Surabaya: Universitas Airlangga Press.
11. Flis L., 2006, *Asymptomatic and Symptomatic Ocular Infections*, Medical University of Lubin, P. 731-732.
12. The United States Pharmacopeia, 2007, *The national Formulary* 25, 30th Edition, Washington, D.C: United States Pharmacopeia Convention Inc. 2928.
13. Departemen Kesehatan RI, 1995, *Farmakope Indonesia* Edisi IV, Jakarta: Departemen Kesehatan RI.