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Ophthalmic Release of in situ gel Ciprofloxacin Hci Based on Combination of Hypromellose and Hci

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Abstract

The development for ophthalmic delivery was purposed to achieve optimum drug loading for ocular therapeutic benefits. An adequate dose of the drug is needed to absorb in the conjunctival sac to take effect. In situ gel preparation was expected to provide these needs with the polymer aid that makes the droplets suddenly coagulate in the eye area to maintain the drug dose. The in situ gel dosage form is desired to overcome the poor bioavailability of conventional ciprofloxacin HCl eye drops on the market. Thus, this work was studied using two cellulose polymers such as hydroxyl propyl cellulose (HPC) and hydroxypropyl methylcellulose (HMPC) as a gelling forming agent. The effect of the in situ ophthalmic quality of the gel due to the two individual polymers separately and their combined use was investigated. The in situ gel quality includes the ability of gel-forming under the influence of varying temperature and stirring frequency difference (as a rheological study) was tested together with the drug release model model. Other ophthalmic preparation quality parameters such as clarity, pH measurement, drug content determination, sterility, and antibacterial activity have been evaluated. However, overall in situ gel formulation developed was of better quality compared to the conventional one. Consideration of the choice of cellulose derivative polymer type is seen to affect the quality of controlled release kinetics models.

Keywords: Ophthalmic gel, Ciprofloxacin HCl, HPMC, HPC, Drug release kinetics

1. Introduction

Ophthalmic delivery systems were considered the attainment and retention of optimum therapeutic levels for the treatment of ocular diseases. Various conventional ophthalmic formulations on the market such as eye drops, suspensions, and ointments have very poor restrain drugs due to their rapid washout during lachrymation in the eyes. Usually applied

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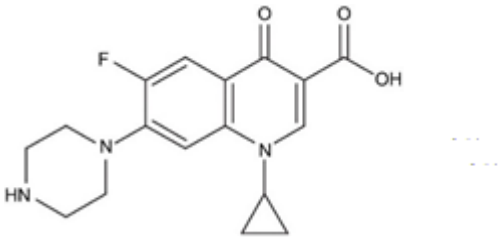
as solutions or suspensions which elimination rapidly observed with ends in poor drug bioavailability. In the case of highly viscous dosage form, such as ointments give blurred vision and patient compliance (Al-Kassas et al., 2009; Dash et al., 2010; Jain et al., 2008; Makwana et al., 2015).

So, an ideal dosage form with convenience and safety for ocular therapy is needed. Especially for an eye infection treatment which needs to care immediately and the precorneal residence time of drugs. For this purpose, designing ophthalmic preparation containing antibiotic should optimize the absorption of the drug and minimize drug loss before penetration the cornea (Rathor, 2010). Recently, various approaches have been reported to delay drug elimination from the conjunctival sac (Kurniawansyah et al., 2018). One of these reports using the hydrogel system based on the concept of in situ gel formation. The system with polymers contains shown ability of sol–to–gel phase transitions due to a specific physicochemical parameter alteration (ionic strengths, pH, or temperature) in the circumstances (Kurniawansyah et al., 2018). The gelation was affected by pH shifting such as cellulose phthalate derivative (Makwana et al., 2015), or by existence cations such as deacetylated gellan gum (Zhu L et al., 2015) and alginate derivate (Al-Kassas et al., 2009; Sharma et al., 2014; Makwana et al., 2015), or by temperature alteration such as poloxamer (Varshosaz et al., 2008; Jain et al., 2008) and (hydroxyl propyl or ethyl or methyl) cellulose derivative (Vigani et al., 2019; Al-Kassas RS et al., 2009; Dash et al., 2010; Jain et al., 2008; Makwana et al., 2015). From all those gelation factors, only thermosensitive which has suitable for the nasolacrimal condition. Therefore, in gel form, the polymers were lowering the drying nasolacrimal and have mucoadhesive properties (Kurniawansyah et al., 2019). Besides those, the application within situ gel was user-friendly, practically easy to prepare, improving therapy efficiency and patient comfort.

In this present work, the preparation and evaluation of in situ gel Ciprofloxacin hydrochloride (CFH) dosage form were conducted. This antibiotic under fluoroquinolone groups commonly used because it has a broad-spectrum antimicrobial activity(Makwana et al., 2015). Effectively proven for ocular infection, such as conjunctivitis and keratoconjunctivitis (Dash et al., 2010). It is highly active for Gram-negative aerobic bacteria including *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus*, and *Neisseriaei* also effective against many Gram-positive aerobic pathogens including penicillinase-producing and methicillin-resistant *Staphylococci* (Makwana et al., 2015). The activity related with inhibiting the DNA girase (topoisomerase II) and topoisomerase IV synthesis of the microorganism (Varshosaz *et al*., 2008). The efficacy of the marketed conventional eye drop in 0.3% solution was restricted by poor bioavailability (Al-Kassas et al., 2009). Then to overcome the problem of the ophthalmic bioavailability of CFH, a polymer with low sensitivity to temperature alternation may select. So, the study of the combination of Hydroxy Propyl Cellulose (HPC) and Hydroxy Propyl Methyl Cellulose (HPMC) as known weak gelling agents at lower

temperatures from each one as in-situ gel ability in eye gel dosage form conducted.

Figure 1. Structure of ciprofloxacin



hydrochloride (Sharma et al. 2010)

MATERIALS AND METHODS

2. Materials

Ciprofloxacin Hydrochloride (CFH) was purchased from Zhejiang Langhua Pharmaceutical Co. Ltd. (China), Hydroxypropyl Cellulose (grade HPC–H) was purchased from Nippon Soda Co., Ltd. (Japan), and Hydroxypropyl methyl Cellulose (grade Metolose 90SH–4000) was obtained from Shin–Etsu Chemical Co. Ltd. (Japan). The bacterials tested were *Bacillus subtilis ATCC* 6633, *Pseudomonas aeruginosa ATCC* 9027, *Staphylococcus aureus ATCC* 25923 and the fungus was *Candida albicans ATCC* 25923. All other chemicals and solvents used were commercially available products of analytical grade.

3. Methods

Compatibility studies

The CFH and the polymers used (HPC and HPMC) as following both compatibilities were checked by FTIR (IR Prestige-21 Shimadzu, Japan). Drug content was detected by UV–Vis Spectrophotometer (SPECORD 200, Analytic Jena, Germany) at a wavelength of 270 nm.

3.1 In Situ Gel Formulations

The developed in situ gel formulations were prepared with various polymers (HPC and HPMC) concentrations as follows in table 1. The ophthalmic in situ gel was prepared as follows. In a different container, HPC and HPMC were dispersed in demineralized water and stirred slowly with a magnetic stirrer. Care was taken to avoid lumps of those polymers during stirring. Then let it sit overnight to swelling in transparency colloids. In another container mix CFH, Benzalkonium Chloride, and NaCl in demineralized water stir until homogenous. Then mix the CFH solution with a polymer solution (HPC or HPMC or a mixture of both) and add PEG 400 stirred until homogeneous. Before adding the remaining demineralized water check the pH of the solution, adjust to pH 4.5 with 0.1 N hydrochloric acids in small increments. If the pH is reached, stir homogeneously.

Table 1. Formulation of ophthalmic in situ gel preparations

Ingredients	Concentrations (% b/v)								
	A1	B1	C1	A2	B2	C2	A3	B3	C3
CFH	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
HPC	0.2	–	0.3	0.3	–	0.2	0.4	–	0.1
HPMC	–	0.2	0.1	–	0.3	0.2	–	0.4	0.3
Benzalkonium Chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Polyethylen Glycol 400 (PEG 400)	1	1	1	1	1	1	1	1	1

NaCl	0.77	0.77	0.86	0.77	0.77	0.86	0.77	0.77	0.86
Aquabidest add …. mL	100	100	100	100	100	100	100	100	100

Description : A : Hydrogel formulation with HPC C : Hydrogel formulation with mixed both HPC and HPMC
B : Hydrogel Formulation with HPMC

3.2 Evaluation of the Formulation

Visual appearances

Checked by observing changes in color, odor, and clarity visually on the day of production and after 28th days of storage.

3.3 pH and Viscosity measurement

The pH measurement of each formulation without any dilution using a pH meter (Hanna®, Japan) was calibrated before use with a buffered solution at pH 4 and 7.

3.4 Analysis of ciprofloxacin

The drug content of ciprofloxacin formulations was determined by dissolving an accurately weighed quantity of 0.1 ml formulation and diluted to 100 ml with phosphate buffer pH 7.4. The solutions were then filtered through a 0.45 m membrane filter and analyzed for ciprofloxacin content by UV–Vis spectrophotometer at 270 nm.

3.5 Sterility Test

The product was sterilized by autoclave at 121ºC for 15 minutes. Conducted with Meanwhile, the viscosity measurements were carried out using Brookfield viscometer model DVII. The developed formulations were placed in the sampler tube using spindle no. 2. To proven the gelling effect by temperature, measurements are carried out at two temperatures namely at room temperature (25ºC) and body temperature (37ºC). For rheological studies, the measurements are measured repeatedly at different speeds on 6, 12, 30, and 60 rpm.

Fluid Thioglycollate (FTM) and Tryptone Soya Broth (TSB) media. Different media are inoculated with different types of microorganisms. The FTM was planted by *Bacillus subtilis ATCC* 6633 and TSB was planted by *Candida albicans ATCC* 25923. Aseptically inoculated directly to each test preparation into a test tube FTM and TSB media and then incubated at 30–35ºC and 20–25ºC, respectively for not less than 14 days. The occurrence of turbidity in the test tube was observed every day.

3.6 Antibacterial Activity

Sample solution (as in situ gel preparations) and standard solutions (as CFH solution) were aseptically filled into each reservoir Petri dishes about 20 µl using a micropipette. In separate Petri dishes were containing bacterial tested *Staphylococcus aureus ATCC* 25923 and *Pseudomonas aeruginosa ATCC* 9027, that has been diluted in Mueller Hinton agar (MHA). Then incubated at 37ºC for 18–24 hours. Measured and recorded diameter clear zone (zone-lysis). Calculate the potential of CFH in all formulation dosage forms.

3.7 In Vitro release studies

In vitro release diffusion tested using phosphate buffer pH 7.4 as STF (Simulated Tear Fluid) for dissolution medium, which is equivalent to the pH of the lachrymal fluid. The in vitro release study was performed by Franz diffusion apparatus

with the speed of rotation maintained at 100 rpm. The apparatus was placed in a water bath to maintained medium temperature (maintained at 37 ± 0.5°C). The samples which were collected from the in vitro diffusion test at various time intervals and analyzed the drug concentration using a UV-Visible Spectrophotometry at 270 nm.

4. RESULTS AND DISCUSSION

The quality of the in situ gel preparation that has been made needs to be observed for evaluation.

4.1 Compatibility studies

The choice of ingredients in the formulation needs to be studied before there is a risk that reduces stability. For this purpose, the FTIR examination of the ingredients to be mixed is examined as shown in Fig. 2.

The FTIR technique was used as a compatibility study between CFH and two polymers utilized. The spectral study of the spectrograph (Fig. 2) and spectrums (Table 2) shown that no interaction indicated by no change in the spectrograph patterns in the drug-polymer mixture. That means the polymer is safe for formulation because it does not change the functional groups of the active pharmaceutical ingredients.

4.2 Evaluation of the formulation

All the formulations prepared quality were evaluated for clarity, pH measurement, viscosity, and drug contents.

4.3 Visual Appearance

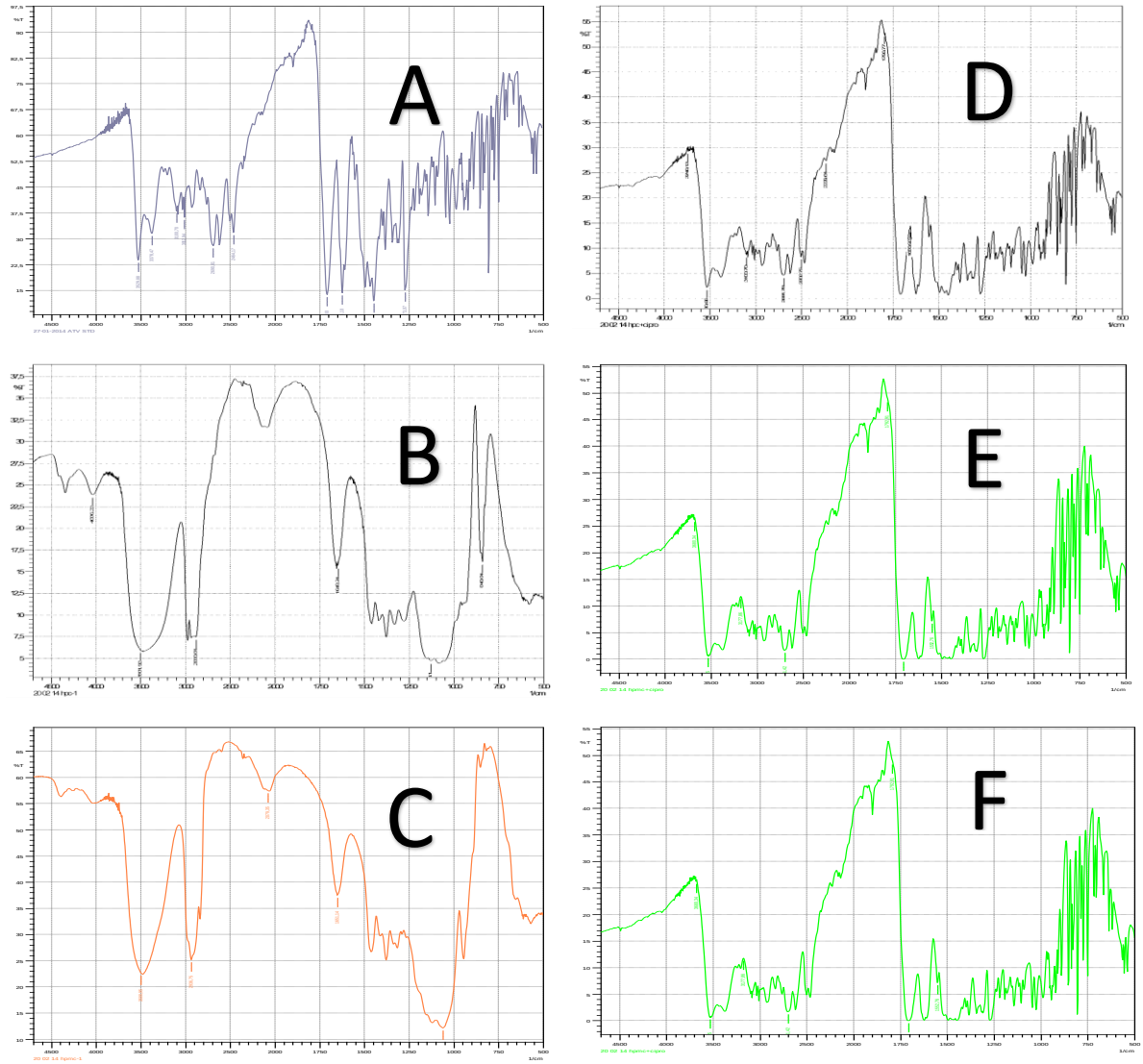
The clarity of visual appearance was conducted by observing the solution against white and black background under fluorescent light. The solution was found to be clear and free from particulate matter. Preparation inspections made from the beginning up to 28 days of storage remain unchanged significantly as shown in Table 3. This shows that the preparation made is quite stable within 28 days of storage.

4.4 pH Measurement and Viscosity

The pH of preparations was adjusted for around 4.5 according to USP monograph (3.5–5.5) and to avoid CFH degradations.

From rheological studies found a decrease in viscosity due to increased speed. The viscosity behavior was described as a pseudoplastic type as illustrated in Fig. 3 and Table 4. The concentration increased of HPC and HPMC did not affect the rheogram profile. Likewise, the combination of both does not give a difference in the rheogram profile.

The viscosity changes may influenced by temperature and pH, the test at the same speed showed that an increase in temperature and pH actually decreases the viscosity of the preparations as shown in Table 5.



Original Material Mixture of Material for preparations

Figure 2. The infrared spectrums of (A) CFH, (B) HPC, (C) HPMC, the combination of both ((D) CFH–HPC and (E) CFH–HPMC) and (F) all of combinations (CFH–HPC–HPMC).

4.5 Sterility Tested

The requirement of ocular dosage form has to be sterile after the final sterilization by autoclave at 121°C for 15 minutes. The sterility tested results that all formulation in sterile after final sterilization as shown in Table 6.

Table 2. The functional groups of FTIR spectrums from CFH and polymers

Functional Groups	**Spectrum IR (cm^{-1})**	**CFH**	**CFH with HPC + HPMC**
O- H	3700- 3500	3529.88	3550.86
N- H	3400- 3300	3378.47	3356.78
C- H aromatic	3020-3000	3100.70	3177.86

O- H carboxylic acid	3400-2400	2690.81	2701.40
C = O	1760- 1690	1739.00	1739.28
C = C	1600- 1470	1638.20	1662.76
C- N	1360- 1250	1273.07	1273.10

1. **Table 3.** Physicochemical Evaluation of Formulations

2.

Visual appearance			pH			Viscosity (cPs)			Drug Contents (%w/v)		
Formu-lations	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
1	Dilute, Clearly, Transparent, Odorless			4.60	4.60	4.55	16	21	29.5	102.70+ 0.06	99.24+ 0.08	100.87+ 0.13
2	Dilute, Clearly, Transparent, Odorless			4.50	4.50	4.60	20.7	22.8	28	100.73+ 0.17	101.61+ 0.13	101.40+ 0.14
3	Dilute, Clearly, Transparent, Odorless			4.50	4.50	4.58	24	25	30.7	101.36+ 0.12	102.47+ 0.11	102.65+ 0.09

Description : A : Hydrogel formulation with HPC
B : Hydrogel Formulation with HPMC
C : Hydrogel formulation with mixed both HPC and HPMC

Table 4. Rheology profile of formulations in various spindle speed

Formu-lations	Viscosity (cPs)														
	20 rpm			30 rpm			50 rpm			60 rpm			100 rpm		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	31.8	33	41	24.5	25	31.5	16	20	25.6	13.9	18	21.8	5.3	19	15.5
2	40.4	58	42.8	31.5	40	33.6	20.7	32	26.8	17.8	24	22.8	7.9	19	16.6
3	46.3	63	45.3	35.9	43	36.2	24	37	28.4	21.3	33	24	9.6	26	18.2

Description : A : Hydrogel formulation with HPC
B : Hydrogel Formulation with HPMC
C : Hydrogel formulation with mixed both HPC and HPMC

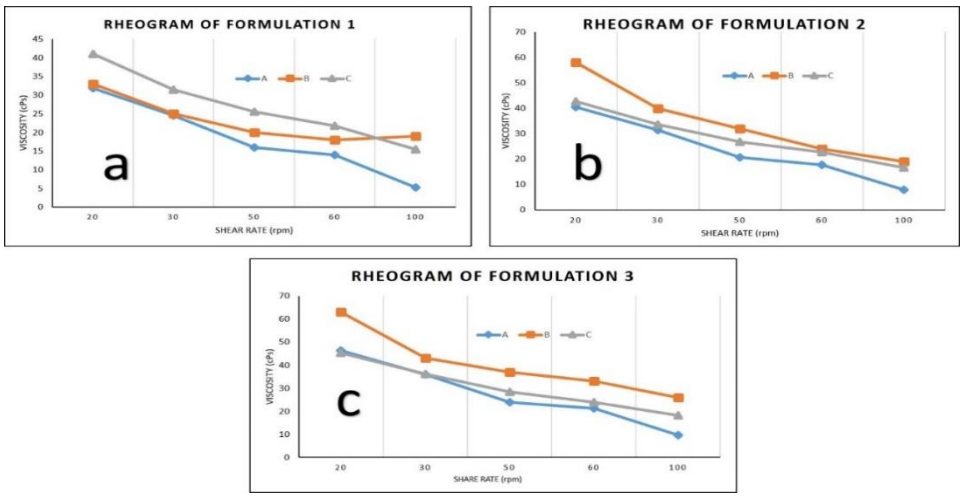


Figure 3. The rheogram profile of formulation :
a) 1 (with HPC 0.2%, HMPC 0.2% and HPC : HMPC = 0.3% : 0.1%)
b) 2 (with HPC 0.3%, HMPC 0.3% and HPC : HMPC = 0.2% : 0.2%)
c) 3 (with HPC 0.4%, HMPC 0.4% and HPC : HMPC = 0.1% : 0.3%)

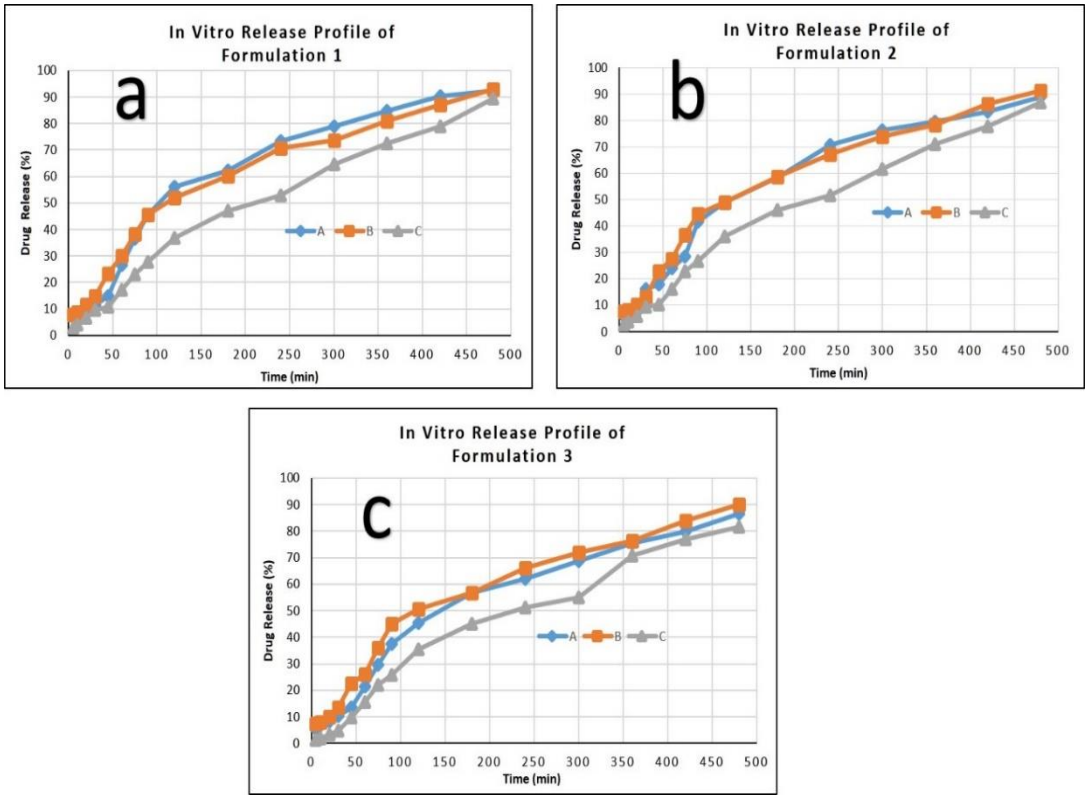


Figure 4. The in vitro release profile of formulation :

a) 1 (with HPC 0.2%, HMPC 0.2% and HPC : HMPC = 0.3% : 0.1%)
b) 2 (with HPC 0.3%, HMPC 0.3% and HPC : HMPC = 0.2% : 0.2%)
c) 3 (with HPC 0.4%, HMPC 0.4% and HPC : HMPC = 0.1% : 0.3%)

4.6 Antimicrobial activity assays

The potency of antimicrobial activity of formulations were carried out against gram positive (*Staphylococcus aureus ATCC* 25923) and gram-negative (*Pseudomonas aeruginosa ATCC* 9027) organisms. It appears that the antimicrobial potency is not greatly influenced by the viscosity or drug release model from the formulation. The comparison of inhibition zones was evaluated to have better activity against gram-negative as shown Table 7.

Table 5. Viscosity measurement in different temperature and pH

Formu-lations	At 25°C						At 37°C (with STF)					
	Viskosity (cPs) 50 rpm			pH			Viskosity (cPs) 50 rpm			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
1	16	5.1	28.7	4.5	4.82	4.55	13	4.5	25	6.5	5.48	6.5
2	21	8.6	27.5	4.5	4.90	4.6	15	7.2	23.6	6.7	5.59	6.65
3	25	14.1	29.2	4.5	4.67	4.5	20	12.7	26.2	6.7	5.18	6.7

Description : A : Hydrogel formulation with HPC
B : Hydrogel Formulation with HPMC
C : Hydrogel formulation with mixed both HPC and HPMC

Table 6. The sterility tested on CFH formulations

Day	Formulations					
	A_{1-3}		B_{1-3}		C_{1-3}	
	FTM	TSB	FTM	TSB	FTM	TSB
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-

Day	Formulations					
	A_{1-3}		B_{1-3}		C_{1-3}	
	FTM	TSB	FTM	TSB	FTM	TSB
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	-	-	-	-	-	-

Description :
+ : Found growth of microorganisms
– : Not found growth of microorganisms

Table 7. Antimicrobial Activity Assays

Formula-tions	Zone of Inhibition (cm) ***Staphylococcus aureus***						Zone of Inhibition (cm) ***Pseudomonas aeruginosa***					
	A	*Eff.* %	B	*Eff.* %	C	*Eff.* %	A	*Eff.* %	B	*Eff.* %	C	*Eff.* %
1	5.06 ± 0.115		5.13 ± 0.040		5.09 ± 0.025		5.13 ± 0.006		5.05 ± 0.035		5.09 ± 0.06	
2	4.98 ± 0.085		5.02 ± 0.080		5.00 ± 0.025		5.10 ± 0.038		5.10 ± 0.095		5.1 ± 0.03	
3	4.93 ± 0.068		5.08 ± 0.145		5.06 ± 0.025		5.06 ± 0.020		5.02 ± 0.02		5.06 ± 0.035	
Avr	4.99 ± 0.064	**91.14**	5.08 ± 0.055	**92.78**	5.05 ± 0.045	**92.24**	5.09 ± 0.038	**98.17**	5.06 ± 0.043	**97.59**	5.08 ± 0.018	**97.97**
Std	5.475 ± 0.175						5.185 ± 0.025					

Description : A : Hydrogel formulation with HPC
B : Hydrogel Formulation with HPMC
C : Hydrogel formulation with mixed both HPC and HPMC
Eff. % : Efficacy in percentage = (Avr / Std) x 100%
Avr : Averages
Std : Standard solution pure of CFH 0.3%

4.7 In Vitro Release Studies

Upon analysis of the correlation coefficient of the percentage of cumulative drug release against a time function as found in Table 8, it appears to have a different trend between separate polymers and their combined use. Hydrophilic polymers such as cellulose derivatives generally provide a model for the release of diffusion drugs concerning transferring the doses from the dosage form to the in vitro medium used (Jain et al. 2008). In the use of a separate polymer, the in vitro release profile of formulation looks an ordinary drug from within a hydrophilic matrix such as first-order kinetics. But in both combinations, the kinetics models transform to non-Fickian transport mechanism as the Korsmeyer-Peppas model. Show that the amount of doses is maintained better in the right combination. These results state that drug release can be controlled by adjusting to a mixture of both in a better composition.

Table 8. Kinetic parameters of in situ gel CFH Formulation

Formulations		Correlation Coefficient (R^2)			
		Zero Order	First Order	Higuchi Model	Korsmeyer Peppas Model
A	1	0.8937	**0.9934**	0.9682	0.9461
	2	0.9122	**0.9912**	0.9793	0.9755
	3	0.9255	**0.9905**	0.9816	0.9658
B	1	0.9111	0.9826	**0.9836**	0.9719
	2	0.9177	**0.9865**	0.9847	0.9658
	3	0.9115	**0.9834**	0.9811	0.9651
C	1	0.9762	0.9671	0.9883	**0.9899**
	2	0.9757	0.9774	0.9880	**0.9887**
	3	0.9644	0.9856	**0.9858**	0.9768

5. CONCLUSION

Overall the formulation developed has fulfilled the requirement of eye drops product as USP monograph. The designed composition has shown the combination of both cellulose polymer derivatives can restrain drug doses better than each polymer. Proven by the release kinetics of the combination formulation was longer, namely Korsmeyer-Peppas model.

6. CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Abdassah, M., Wardhana, Y.W., Rusdiana, T., Yordan, M.Z.A., Anggraini, F.D., Tedjasaputra, M.G. (2016). Kinetic Study on Extended Release of Theophylline Caplet with Different Brands Hypromellose Matrix. *Bulletin of Pharm Res* 6(1), 1–6.
2. Al-Kassas, R.S., El-Khatib, M.M. (2009). Ophthalmic Controlled Release In Situ Gelling Systems for Ciprofloxacin Based on Polymeric Carriers. *Drug Delivery* 16(3), 145–152.
3. Dash, S., Murthy, P.N., Nath, L., and Chowdhury, P. (2010). Kinetic Modeling on Drug Release From Controlled Drug Delivery Systems. *Acta Poloniae Pharmaceutica–Drug Research* 67(3), 217–223.
4. Jain, S.P., Shah, S.P., Rajadhyaksha, N.S., Singh, P.S.P.P., Amin, P.D. (2008). In Situ Ophthalmic Gel of Ciprofloxacin Hydrochloride for Once a Day Sustained Delivery. *Drug Development and Industrial Pharmacy* 34, 445–452.
5. Kurniawansyah, I.S., Rusdiana, T., Wahab, H.A., Subarnas, A. (2019). *In Situ* Ophthalmic Gel With Ion Activated System. *Int J App Pharm* 11(4), 15–18.
6. Kurniawansyah, I.S., Sopyan, I., Wardhana, Y.W., and Gunasekaran, M. (2018). Formulation and Evaluation of Chloramphe-nicol Hydrogel Ophthalmic Preparation *J Young Pharm* 10(2) Suppl, s73–78.
7. Kurniawansyah, I.S., Sopyan, I., Wathoni, N., Fillah, D.L., Praditya, R.U. (2018). Application and Characterization of *In Situ* Gel. *Int J App Pharm.* 10(6), 34–37.
8. Makwana, S.B., Patel, V.A., and Parmar, S.J. (2015) Development and Characterization of In-Situ Gel for Ophthalmic Formulation

Containing Ciprofloxacin Hydrochloride. *Results in Pharma Sciences* 6, 1–6.
9. Ministry of Health RI. Indonesian Pharmacopoeia. 5th ed. Jakarta. 2014
10. Rathor, K.S. (2010). In Situ Gelling Opthalmic Drug Delivery System: An Overview. *Int J Pharm Pharm Sci* 2, 30–34.
11. Rowe, R.C., Sheskey, P.J., and Quinn, M.E. (2009). *Handbook of Pharmaceutical Excipients*. Sixth Edition. London: Pharmaceutical Press. 317–319.
12. Sharma, A., Sharma, J., Kaur, R., and Saini, V. (2014). Development and Characterization of *In Situ* Oral Gel of Spiramycin. *BioMed Research International* Vol. 2014, 1–7.
13. Sharma, P.C., Jain, A., Jain, S., Pahwa, R., and Shahar, Y.M. (2010). Ciprofloxacin: review on developments in synthetic, analytical, and medicinal aspects, *Journal of Enzyme Inhibition and Medicinal Chemistry* 25(4), 577–589.
14. The United States pharmacopeia and National Formulary (USP 32 – NF 27) (2009). Rockville (MD): United States Pharmacopeial Convention, Inc.
15. 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.
16. Vigani, B., Faccendini, A., Rossi, S., Sandri, G., Bonferoni, M.C., Gentile, M., and Ferrari, F. (2019). Development of a Mucoadhesive and In Situ GellingFormulation Based on κ-Carrageenan for Applicationon Oral Mucosa and Esophagus Walls. I. A Functional In Vitro Characterization. *Mar Drugs* 17(112), 1 – 16.
17. Zhu, L., Ao, J., and Li, P. (2015). A Novel in Situ Gel Base of Deacetylase Gellan Gum for Sustained Ophthalmic Drug Delivery of Ketotifen: In Vitro and in Vivo Evaluation. *Drug Des Devel Ther* 9, 3943–39