

STUDY OF ISOTONICITY AND OCULAR IRRITATION OF CHLORAMPHENICOL *IN SITU* GEL

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ABSTRACT

Objective: The objective of this study was to find out the isotonicity of chloramphenicol ophthalmic *in situ* gel and to know the irritating effect of its in the eyes of test animals, so it can be to maximize absorption of the drug in the eye, minimize drug loss before corneal penetration and safe to used.

Methods: This study were started by making four aseptic formulations of *in situ* gel preparations with a comparison of the baseline concentrations of different Poloxamer 407 and HPMC, F1 (5: 0.45), F2 (10: 0.45), F3 (5: 1) and F4 (10: 1). Four aseptic of *in situ* gel preparations, followed by a qualitative isotonicity test using blood cells to see the comparison between control and test preparations, and ocular irritation test using the draize test method to determine the presence or absence of the irritation.

Results: The results obtained from the isotonicity test showed that the four preparations have normal blood cells that similar with isotonic control solution; therefore, it can be said that the preparations have been made isotonic.

The results of the ocular irritation test using the draize test method showed for each category, such as cornea, iris, conjunctiva and edema were zero. A zero value on the cornea indicates no ulceration or opacity, and the iris, conjunctiva and edema were normal. **Conclusion:** Chloramphenicol *in situ* gel are isotonic and do not cause irritation to the rabbit's eyes, so they are safe to use and the formulation can be used for further research until the final goal is obtained.

Keywords: Chloramphenicol, HPMC, In situ gel, Poloxamer 407, Isotonicity, Ocular irritation, draize test

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INTRODUCTION

Ophthalmic drug delivery system is one of the most interesting and challenging endeavours facing pharmaceutical scientists [1, 2]. The use of the new gel system, which is instilled as drops into the eye and undergo a sol-gel transition in the cul de sac can overcome the problem of poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug [2, 3]. Another advantage of *in situ* gel is easy to use, simple manufacturing at the factory, and improve both adherence and patient comfort by minimizing the frequency of its use [4].

Characterization of *in situ* gel was determined to ensure that the prepared preparation met the standard and it safe. Isotonicity is an important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of eye [3, 5]. Isotonic solutions maintain the integrity of blood cells, whereas hypertonic shows shrinkage and hypotonic shows the bulging of cells [6]. Assessment of the ocular irritation potential of ophthalmic solutions represents an extremely important step in the development of both over-the-counter and prescription pharmaceuticals [7].

The objective of the research work was to find out the isotonicity of chloramphenicol *in situ* gel ophthalmic preparations and to know the irritating effect of ophthalmic gel *in situ* chloramphenicol gel in the eyes of test animals.

MATERIALS AND METHODS

Ethical clearance application

Request for ethical clearance from the Health Research Ethics Commission, Faculty of Medicine, Universitas Padjadjaran.

Chemicals

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

Table 1: 2² factorial design optimization results of chloramphenicol ophthalmic *in situ* gel

Chemicals	Formulas of chloramphenicol <i>in situ</i> gel (% 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

*Amount of Benzalkonium chloride in terms of % v/v, Note: F1–F4 = Formula 1–Formula 4

Optimization of ophthalmic chloramphenicol *in situ* gel formula

The optimization was performed on the poloxamer 407 and HPMC bases by using a 2² factorial design. The concentration of poloxamer 407 (X1) and concentration of HPMC (X2) were chosen as independent variables in 2² full factorial designs. The real values at the lower and upper levels of X1 were 5% and 10% and X2 were 0.45% and 1%.

Formulation of ophthalmic chloramphenicol *in situ* gel

The optimization of the formula was performed on poloxamer 407 and HPMC bases by using a 2² factorial design. Optimization with a two-level factorial resulted in 4 variations of the formulas, as listed in table 1.

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

Procedure for chloramphenicol *in situ* gel formulation

The formulation process has been carried out aseptically in a laminar airflow (LAF) room that was sterilized with 70% alcohol. After cleaning, the UV lamp was turned on for 1.5 h. After that, the neon light and the blower were turned on. Each formula was made aseptically. The poloxamer 407 and HPMC bases were made by dissolving each substance in hot aqua dest in a separate container. The poloxamer 407 was dissolved continuously and gradually with slow stirring to prevent the formation of foam. It was dissolved in an aqueous phase that has been heated to 70–90 °C [8]. The HPMC base was made by putting the required amount of hot water (approximately 70 °C) into the container. HPMC was added gradually and we waited for it to float on the surface of the water. After each polymer was dissolved, and the two polymers were mixed and stirred until homogeneous. The mixed base was cooled to support the gelling process. Then the mixed base was sterilized with an autoclave at 121 °C for 15 min [9]. The autoclave sterilized base was mixed with a mixture of chloramphenicol, propylenglycol and benzalkonium chloride that had been sterilized before with a bacterial filter (0.22 µm) until homogeneous.

Isotonicity evaluation

Isotonicity is an important characteristic of the ophthalmic. Isotonicity has to be maintained to prevent tissue damage or irritation of the eye. The optimized formulations were mixed with few drops of blood and then the shape of blood cells was observed in the microscope under ×45 magnifications [10-12]. The results obtained were compared with the control solution. Isotonic solutions maintain the integrity of blood cells, whereas hypertonic shows shrinkage and hypotonic shows the bulging of cells.

Ocular irritancy

The Draize technique was designed for the ocular irritation potential of the ophthalmic product before marketing. One hundred

microliters of the formulation were placed into the lower cul-de-sac with an observation of the various criteria made at the designed required time interval of 1 h, 24 h, 48 h, 72 h after administration [12-14]. Approval of the Institutional Animal Ethics Committee was obtained before the commencing of the study. Ethical approval has been given from the Faculty of Medicine Universitas Padjadjaran with Number: 590/UN6. KEP/EC/2019. A total 3 albino New Zealand rabbits (of either gender) weighing 1.5–2 kg were used for the present study. Three rabbits are used for the four formulas, which will be given treatment, namely the preliminary test and the confirmation test for each formula. Prior to the test, the rabbits were acclimatized for 5 d with the cleaned condition and they eat normally, and then their eye health was confirmed. Then a preliminary test was carried out using one rabbit first by exposing the preparation to one rabbit's eye and the other eye as a negative control. In this preliminary test, the eyes of the rabbits were observed at the 1st, 24th, 48th and 72nd hours after exposure to the test preparation, after which confirmation tests were carried out on two other rabbits. These two tests were performed for all formulas. Retrieval of irritation data on experimental animals will be taken photos along with the score data adjusted to the Regulation of the Head of BPOM Number 7 of 2014 [15, 16].

The assessment of the reaction of the test preparation against the eye is assessed and recorded according to the score stated in the regulation. Assessment of eye reactions (conjunctiva, cornea, iris) and other injuries to the eye or systemic effects must be reported.

The eye irritation score that should be evaluated is the degree of eye injury and the presence or absence of reversibility. Individual scores do not represent an absolute standard for the irritant properties of the test preparation but should be viewed as a reference value. The test preparation irritation score is a combination of all observations from the test [16-18].

RESULTS

Isotonicity of *in situ* gel preparations

After observing using a microscope, the results showed that the four preparations have given a normal blood cell that similar results with isotonic control solution. This can be seen in fig. 1 and 2 where observations were made using a isotonic control solution in the form of NaCl 0.9%, a hypertonic control solution in the form of NaCl 2% and hypotonic control solution in the form of NaCl 0.2%. Therefore it can be said that the preparations have been made isotonic.

Ocular irritation test results using the draize test method

Ocular irritation testing was carried out using rabbits that have received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University with Number: 590/UN6. KEP/EC/2019. The degree of irritation was evaluated by scoring the irritation that occurs in the cornea, iris, conjunctiva and edema. The data can be seen in table 2.

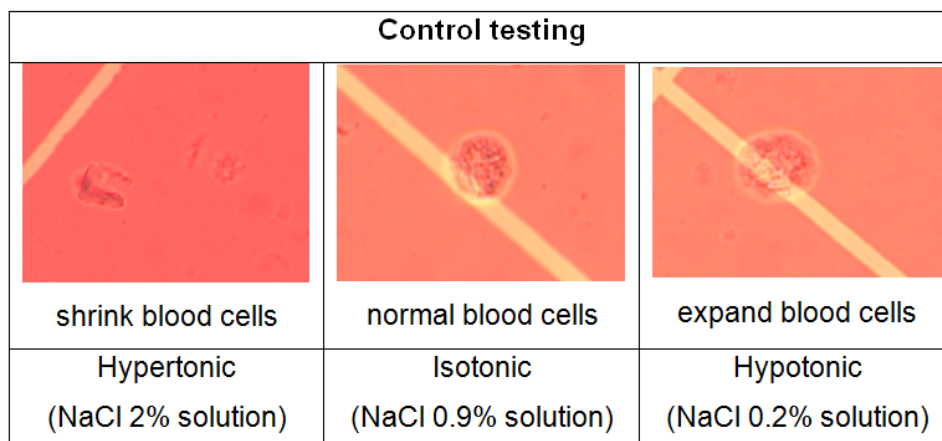


Fig. 1: Observation results of isotonicity test on control solution

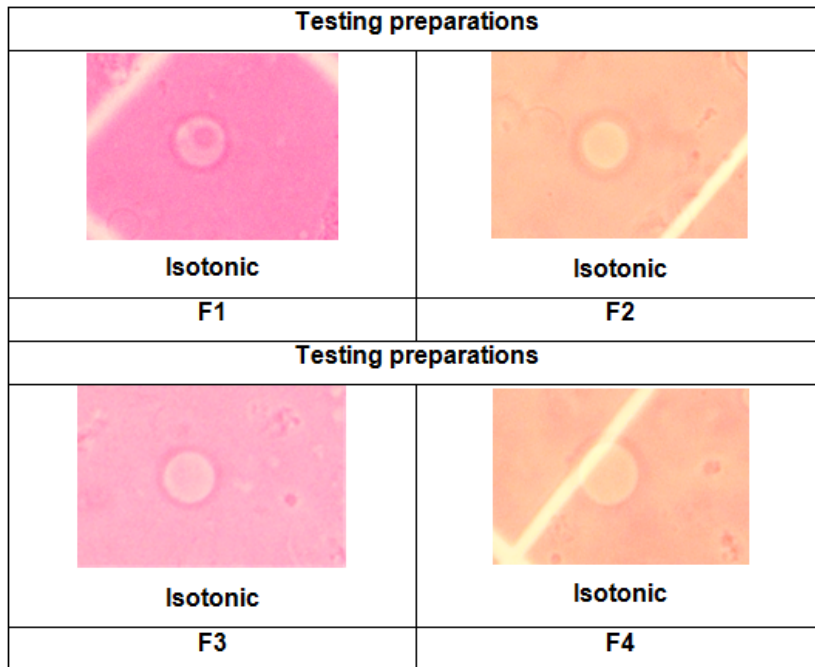


Fig. 2: Observation results of isotonicity test on preparations solution

Table 2: The score of irritation

	Rabbit 1 (preliminary test)				Rabbit 2 (a confirmation test)				Rabbit 3 (a confirmation test)			
	Cr	I	Cj	E	Cr	I	Cj	E	Cr	I	Cj	E
F1	0	0	0	0	0	0	0	0	0	0	0	0
F2	0	0	0	0	0	0	0	0	0	0	0	0
F3	0	0	0	0	0	0	0	0	0	0	0	0
F4	0	0	0	0	0	0	0	0	0	0	0	0

Cr: Cornea, I: Iris, Cj: Conjunctiva, E: Edema

Based on the observations made, the results obtained for each category, such as cornea, iris, conjunctiva and edema were zero. The purpose of this zero value from each category has its own description. A zero value on the cornea indicates no ulceration or

opacity, the iris indicates normal, the conjunctiva shows normal blood vessels or no redness that mean normal, and the edema category a zero value indicates normal that there is no swelling in the rabbit's eye [16-18].

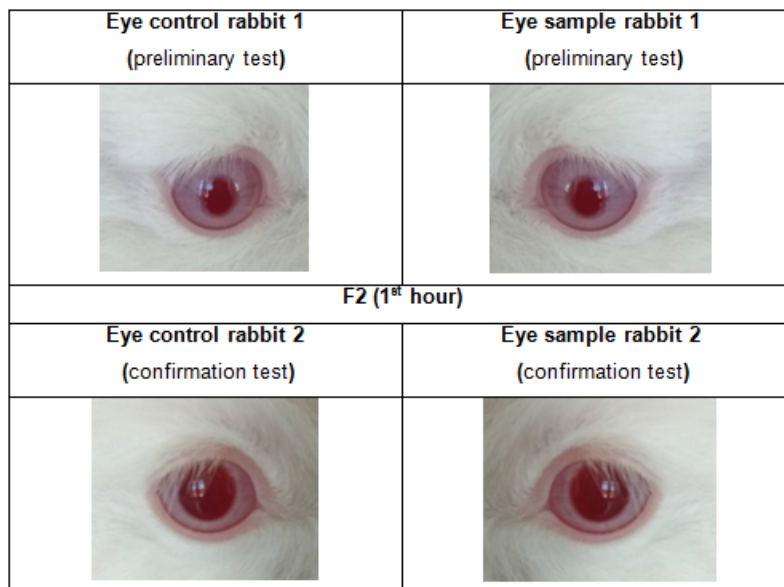


Fig. 3: Example image of the result of irritation test on rabbit's eye

If seen from the results listed in table 2 and fig. 3, it can be said that the preparations made do not cause irritation to rabbits. In the irritation test above, a preliminary test was carried out using a rabbit, which according to the Regulation of the Head of BPOM Number 7 of 2014 the purpose of this preliminary test is to determine whether the test preparation is a strong irritant or not. If the preliminary test shows that the preparation is a strong irritant, no further testing should be carried out. If in the preliminary test the preparation does not indicate

a strong irritant, a confirmation test must be carried out using two additional animals. In the preliminary test and confirmation test, observations were made at the 1st hour, 24th h, 48th h and 72th h after the exposure of the test preparation, after which an assessment was carried out with the scores shown in table 3. The extrapolation of the results of eye irritation testing in animals to humans can be considered valid because in some cases, albino rabbits are known to be more sensitive than humans in terms of eye irritation [17, 18].

Table 3: Grading of ocular lesions [18]

Object of observation	Score
Cornea: Opacity: degree of density (readings should be taken from most dense area)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Maximum possible: 4	
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect)	1
Hemorrhage, gross destruction, or no reaction to light	2
Maximum possible: 2	
Conjunctiva	
Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse beefy red	3
Maximum possible: 3	
Chemosis/edema Swelling (refers to lids and/or nictating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half-closed	3
Swelling, with lids more than half-closed	4
Maximum possible: 4	

This eye irritation test aims to obtain information on the dangers that arise when the test preparation is exposed to the eyes and the mucous membranes of the eyes. In the test, a preliminary test was carried out with one rabbit first, then followed by a confirmation test with two other rabbits.

The *in situ* gel preparation formulation was obtained from the optimization results that were carried out in previous studies using a two-level factorial method in the Design Expert® software. The formula used has the active substance chloramphenicol, which is a broad-spectrum antibiotic that actively works on many types of microbes, namely gram-positive bacteria and gram-negative bacteria. In a study conducted by Nayak *et al.*, the chloramphenicol concentration of 0.5% in ophthalmic preparations is a concentration that has proven its effectiveness in the treatment of eye infections and chloramphenicol with a concentration of 0.5% is an eye drop preparation that is usually present in the market [19].

This formula used two bases, Poloxamer 407 and HPMC which are responsive to sol-to-gel phase changes. Poloxamer 407 undergoes a sol-to-gel phase transition due to an increase in temperature which causes poloxamer 407 to gradually desolvate the polymer and experience an increase in micelle aggregation. This process eventually causes the formation of micelles due to the dehydration process of the polyoxypropylene blocks from the poloxamer, then the micelles touch each other and no longer move. However, the mechanical strength of poloxamer is weak; therefore poloxamer is combined with HPMC [20, 21].

The use of HPMC together with other polymers (in this study combined with poloxamer) serves to reduce the concentration of poloxamer so that HPMC can stabilize the concentration of poloxamer [21]. However, if the use of a single HPMC (without adding other polymers) functions as a viscosity enhancer, that can

help control the release of preparation [22]. The advantage of the combined use of poloxamer 407 and HPMC bases is that this combination increases the viscosity of the preparation so that the contact time of the preparation with the eye is longer so that it can increase the bioavailability of the drug [21, 23].

CONCLUSION

All formulations of chloramphenicol *in situ* ophthalmic gel prepared showed good isotonicity that the preparations have been made isotonic, and did not cause irritation to the eyes of rabbits, so the preparation was safe to use and the formulation can be used for further research until the final goal is obtained.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest

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