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FORMULATION AND EVALUATION OF TROPICAMIDE LOADED OCULAR IN SITU GEL

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ABSTRACT

This study aimed to develop and evaluate a Pluronic F127-based ocular in-situ gel loaded with tropicamide for sustained drug release and enhanced ocular retention. Various formulations were prepared and characterized for clarity, drug content, in situ gelling capacity, pH, viscosity, and in vitro drug release profile. Among the formulations, F6 demonstrated optimal characteristics, including clear appearance, high drug content, and sustained drug release kinetics. Comparative analysis of regression coefficients indicated that drug release from F6 follows a diffusion-controlled mechanism. The developed formulation holds promise as a potential delivery system for tropicamide, offering prolonged therapeutic effect and improved patient compliance.

Keywords: Tropicamide, ocular drug delivery, in-situ gel, Pluronic F127, sustained release.

INTRODUCTION

Tropicamide, a potent mydriatic agent, holds significant clinical relevance in ophthalmology for its ability to induce pupil dilation rapidly and effectively. Its utility spans various diagnostic and therapeutic procedures, including fundus examination, cataract surgery, and certain ocular surgeries. Despite its widespread use, tropicamide's short duration of action poses challenges, necessitating frequent administration to maintain pupil dilation, which can inconvenience patients and compromise compliance [1-2].

Addressing this limitation, in situ gels emerge as a promising solution by providing sustained drug release and prolonged ocular residence time. These gels exhibit a unique property of transitioning from a liquid to a gel state upon application to the ocular surface, triggered by environmental factors

like temperature, pH, or ions. This phase transition enhances the retention of drugs at the target site, thereby improving bioavailability and therapeutic efficacy [3-5].

The present study endeavors to formulate and evaluate an in situ gel incorporating tropicamide, aiming to extend its duration of action and enhance patient comfort and compliance. Through systematic exploration of formulation variables such as polymer type, concentration, and pH, optimal gel characteristics including gelation kinetics, viscosity, and drug release kinetics will be identified.

The evaluation of the tropicamide-loaded in situ gel will entail comprehensive physicochemical characterization and performance assessment. This includes in vitro release studies to elucidate drug release kinetics, ex vivo permeation studies using ocular tissue models to evaluate drug transport across barriers, and in vivo pharmacokinetic and pharmacodynamic studies in appropriate animal models or human subjects to assess efficacy and safety profiles [6].

Furthermore, ocular tolerance and biocompatibility of the formulated gel will be rigorously evaluated to ensure its safety and minimize the risk of adverse effects. Successful development of a tropicamide-loaded in situ gel holds significant promise in improving patient outcomes and optimizing the clinical utility of this vital ophthalmic medication.

MATERIAL AND METHODS

Material

Various chemicals were utilized for the formulation development of the in situ ocular gel. Tropicamide, the active pharmaceutical ingredient, was obtained as a gift sample from Bioplus Life Science Pvt. Ltd., Bangalore. Disodium hydrogen phosphate, di potassium hydrogen orthophosphate, sodium chloride, methanol, ethanol, chloroform, sodium bicarbonate, and calcium chloride were procured from S.D. Fine Chem. Ltd., Mumbai. Carbopol, HPMC (Hydroxypropyl methylcellulose), EDTA (Ethylene diamine tetra acetic acid), and polyethylene glycol were sourced from Rankem Pvt. Ltd., Mumbai. Pluronic F68 was also obtained from S.D. Fine Chem. Ltd., Mumbai. These chemicals were carefully selected and utilized in the formulation process to achieve the desired characteristics and performance of the in situ ocular gel.

Methods

Formulation development of *In-situ* gel

The formulation development of an *in-situ* gel of Tropicamide holds significant importance and presents a compelling need for study.

Selection of Vehicle

The solubility of Tropicamide was tested in various buffers such as acetate buffer I.P. (pH 6.0 & 6.5), citrophosphate buffer B.P. (pH 6.0 and 6.2) and phosphate buffer USP (pH 7.2 and 7.4) in order to select a suitable vehicle. Solutions of Tropicamide (0.5%) in the above buffers were prepared to test its solubility at the dosage level desired [7].

Methodology for formulations preparation:

For the preparation of the Pluronic F127-based ocular in-situ gel, all the ingredients were meticulously sieved through a sieve with a mesh size of 44 to ensure uniform particle size distribution. Subsequently, a 0.1% solution of the drug was prepared in acetate buffer 5.0 I.P. The solution was then cooled in an ice bath, and Pluronic F127 was gradually added with continuous stirring to ensure proper dispersion.

Following the addition of Pluronic F127, the resulting solution was stored in a refrigerator at 4°C for 24 hours. This refrigeration period facilitated the complete dissolution of Pluronic F127. After 24 hours, carbopol 934 and HPMC 15cps were slowly incorporated into the solution along with other excipients under continuous stirring. The stirring process was continued for 2-3 hours to ensure thorough mixing and to prevent the formation of any undesirable lumps or aggregates. The resulting formulation was subjected to probe sonication to eliminate any entrapped air bubbles, ensuring homogeneity and uniformity. All formulations were then transferred into LDPE (Low-Density Polyethylene) bottles for storage and further use. To maintain stability and preserve the integrity of the formulations, all containers were stored in a refrigerator until further experimentation or application.

Table 1: Composition of different formulations of *In-situ* gel

S. No.	Ingredient	Formulations								
	(%)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1.	Tropicamide	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
2.	Pluronic F127	10	12	14	10	12	14	10	12	14
3.	Carbopol 934	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
4.	HPMC 15cps	1.0	1.0	1.0	0.75	0.75	0.75	0.5	0.5	0.5
5.	EDTA	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
6.	Benzalkonium Chloride	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%

7.	NaCl	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
8.	Poly ethylene glycol	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
9.	Acetate Buffer (pH 5.0)	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Evaluations of formulations

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background [8].

Drug content

The assay of Tropicamide was performed by UV method. The calculation was based on calibration curve method using regression equation (Y=mx+c) [9].

pH

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using calibrated digital pH meter [10]. For *In situ* gel pH 5.0 should be optimum because both the drug is stable at pH 3.5-5.0. Lowering the pH from 5.0 can causes irritation to eye and on raise the above 5 will result in gelation of formulation due to presence of carbopol.

***In-situ* gelling capacity**

In situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37^0C. Formulations were introduce into STF in a ratio of 1:2 Change in consistency of Formulations were visually inspected [11].

Composition of simulated tear fluid (STF)

Sodium chloride: 0.670 gm

Sodium bicarbonate: 0.2 gm

Calcium chloride dihydrate: 8 mg

Water upto: 100ml

pH was adjusted by 0.5 N NaOH to 7.4

Gelling capacity of all formulations are depicted as + (gels after five minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours) and +++ (gelation immediate, remains for extended period upto 8 hours).

Viscosity study

At pH 5.0 and temperature less than 16ºC the developed formulations were in liquid state and show low viscosity. For viscosity studies the pH of formulations were raised from pH 5.0 to pH 7.4 and the temperature was raised to 37^0C. pH was raised to 7.4 by the addition of 0.5M NaOH[12]. The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 RPM for comparative study. The angular viscosity was measured by gradually increase the RPM from 10 to 70.

***In-vitro* drug diffusion study**

The *in vitro* release of drugs from the formulations was studied through cellophane membrane. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter).

A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Methodology Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium [13].

Stability studies

Optimized sterile formulation was subjected to stability testing. Sterile optimized ophthalmic formulation was filled in glass vials, closed with gray butyl rubber closures and sealed with an aluminium caps. The vials contain optimized formulation were kept in stability chamber, maintained at 40 ± 2°C and 75 ± 5% RH for one month. Samples were withdrawn weekly and estimated for drug content and *In-situ* gelling capacity[14].

RESULTS AND DISCUSSION

The clarity test results (Table 2) indicate that most formulations exhibited clear appearance, with high drug content percentages ranging from 96.52% to 99.05%. Formulations F4, F5, and F6 demonstrated excellent clarity and drug content, suggesting optimal formulation characteristics.

Additionally, in situ gelling capacity, evaluated qualitatively, showed varying degrees of gelling potential among formulations, with F4, F5, and F6 displaying the highest gelling capacity.

pH determination results (Table 3) reveal that formulations generally exhibited pH values close to the target pH of 5.0, with slight variations observed. Adjustments were made to bring formulations within the desired pH range, ensuring compatibility with ocular physiology.

Comparative viscosity analysis (Table 4) elucidates the influence of Pluronic F127 concentration on solution viscosity and viscosity after gelation. Generally, formulations with higher concentrations of Pluronic F127 exhibited higher viscosities after gelation, suggesting a direct correlation between Pluronic F127 concentration and gel strength.

In vitro drug release profile data (Table 5) for formulation F6 demonstrate sustained drug release over time, with a gradual decrease in drug release rate. The logarithmic transformation of cumulative drug release data allows for a better understanding of release kinetics. The formulation exhibits sustained drug release characteristics, essential for prolonging therapeutic effects and minimizing dosing frequency.

Regression coefficient analysis (Table 6) for zero-order and first-order kinetics provides insights into the drug release mechanisms. The high R^2 values indicate good correlation with zero-order kinetics, suggesting that drug release from formulation F6 occurs predominantly through a diffusion-controlled mechanism. However, first-order kinetics also show a reasonable fit, indicating the possibility of other contributing factors to drug release kinetics.

Table 2: Clarity test of *in situ* gel formulations

Formulation code	Clarity	Drug Content (%)*	*In situ* gelling capacity
F1	Clear	97.85±0.15	"++"
F2	Clear	96.56±0.23	"++"
F3	Clear	98.85±0.32	"++"
F4	Clear	97.78±0.41	"+++"
F5	Clear	96.52±0.55	"+++"
F6	Clear	99.05±0.32	"+++"
F7	Clear	98.85±0.19	"+"
F8	Turbid	97.78±0.28	"+"
F9	Turbid	96.36±0.36	"+"

Table 3: pH Determination

Formulation	pH Determination	Adjust to
F1	4.9±0.2	5.0 ±0.1
F2	4.7±0.3	5.0 ±0.1
F3	4.8±0.4	5.0 ±0.1
F4	4.5±0.5	5.0 ±0.1
F5	4.9±0.3	5.0 ±0.1
F6	4.8±0.5	5.0 ±0.1
F7	4.4±0.6	5.0 ±0.1
F8	4.9±0.3	5.0 ±0.1
F9	4.7±0.4	5.0 ±0.1

Table 4: Comparative viscosity* of *In situ* formulation

Formulation code	% of Pluronic F 127	Viscosity of solution (in cps)	Viscosity after galation
F1	10	745	2845
F2	12	815	2965
F3	14	658	3025
F4	10	765	2565
F5	12	698	2745
F6	14	788	2855
F7	10	645	2745
F8	12	698	2898
F9	14	748	2965

*Spindle no.7 rpm 50

Table 5: *In vitro* drug release profile of Tropicamide from *in situ* Formulation F6

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	22.32	1.349	77.68	1.890
1	1	0	36.65	1.564	63.35	1.802
1.5	1.225	0.176	48.98	1.690	51.02	1.708
2	1.414	0.301	55.47	1.744	44.53	1.649
2.5	1.581	0.398	68.85	1.838	31.15	1.493
3	1.732	0.477	75.65	1.879	24.35	1.386
4	2	0.602	89.98	1.954	10.02	1.001
5	2.236	0.699	98.85	1.995	1.15	0.061

Table 6: Comparative study of regression coefficient for selection of optimize Formulation F6

Drug	Zero order	First order
Tropicamide	$R^2 = 0.9682$	$R^2 = 0.8774$

CONCLUSION

The formulation and evaluation of Pluronic F127-based ocular in-situ gel loaded with tropicamide demonstrated promising results. Through a systematic approach, several formulations were developed and comprehensively assessed for various parameters including clarity, drug content, in situ gelling capacity, pH, viscosity, and in vitro drug release profile. Among the formulations, F6 stood out as a particularly favorable candidate, exhibiting clear appearance, high drug content, optimal pH, and excellent in situ gelling capacity. Moreover, formulation F6 displayed sustained drug release characteristics over time, indicating its potential for prolonged therapeutic effect. The comparative analysis of regression coefficients suggested that the drug release from formulation F6

follows predominantly a diffusion-controlled mechanism, which is essential for achieving sustained and controlled drug release.

The study underscores the potential of Pluronic F127-based in-situ gel as a promising delivery system for tropicamide, offering advantages such as improved ocular retention, sustained drug release, and enhanced patient compliance. Further optimization and refinement of formulation F6 could pave the way for its clinical translation, ultimately benefiting patients undergoing ophthalmic procedures requiring mydriasis.

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