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Development and Evaluation of Ophthalmic Drop and *In-situ* Gel from Roots of *Boerhaavia diffusa*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AV and VKL designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author Pooja managed the literature searches, analyses of the study performed the spectroscopy analysis and author Pooja managed the experimental process. Author VKL identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation deals to evaluate efficacy of ophthalmic drop and *in-situ* gel formulations of aqueous distillate of *Boerhaavia diffusa* roots using polymers sodium alginate (SA), HPMC (hydroxyl propyl methyl cellulose) 15 cps and Carbopol 940 (CB). Where (SA) was used for eye drop and two in situ gels of aqueous distillate, one was using HPMC (hydroxyl propyl methyl cellulose- 15 cps. And SA (sodium alginate) and another were using HPMC (hydroxyl propyl methyl cellulose- 15 cps and CB (Carbopol 940). Eye drops and gels were sterilized and assessed for various parameters like clarity, pH, physical appearance, physical stability, viscosity and uniformity of drug content. The release rate from the formulation within a period of 9 h for eye drop was 80.56% and in two *in-situ* gels were 84.66% and 82.4% respectively. Both eye drop and ophthalmic gels obeyed zero order kinetics for drug release. The ocular irritation was carried out on male Wister rats and no redness, inflammation and increase in tear was seen. The efficacies of both formulations (eye drop and gel) were assessed using subcutaneously 0.01% w/v sodium

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selenite-induced cataract in male Wister rats. The result concludes that *in-situ* gel is more efficacious than eye drop, and was found to be more stable at ambient, refrigerator and incubated temperature. The stability of eye drop and the gels was evidenced by the degradation rate constant. Ophthalmic gel formulated by HPMC with SA and HPMC with CB, proves to be viable alternative to conventional eye drops as it offers longer precorneal residence time and excellent ocular tolerance.

Keywords: Cataract; ophthalmic drop; in-situ gel, Boerhaavia diffusa; Itone; Carbopol 940 (CB); hydroxy propyl methyl cellulose (HPMC); sodium alginate (SA).

1. INTRODUCTION

In the Ayurvedic system of medicine, as mentioned in ancient Indian books like *Charka Samhita,Sushrut Samhita, Bhav prakasha, Ras Tarang*, Nayan Drastam and *Astanghriday,* there are a number of plants which are used in ophthalmic disorders, either single or in compound formulations. In Ayurveda (Indian system of medicine) various eye disorders and diseases like *Abhishyand* (Conjunctivitis), *Adhimanth* (Glaucoma), *Timir* (Cataract), etc. have been described in great details [1]. Their etiology and treatments have also been described.

In Ayurvedic terminology, cataract is termed *Linga Nasha* or *Timira*. According to Ayurvedic principles, such a condition develops due to the aggravation of Vayu. Here, aggravation of Vayu dries up the liquid that makes the lens and the retina supple [2].

In general, cataract is the opacification (light impenetrability) of the lens. In this condition, the lens of the eyes interferes with the eye vision**.** Oxidation of lens proteins SH- groups induce protein conformational changes leading to protein aggregation and opacification of the lens resulting in block of light transmission to the retina and then blindness [1,2,3].

Na+K+ATPase also play an important role in maintaining the lens transparency and its alteration is one of the major events leading to cataract formation. Impairment of Na+ ATPase activity causes accumulation of Na+ and loss of K+ with hydration and swelling of the lens fibers leading to cataractogenesis. [4] reduced glutathione (GSH) was found to be depleted in diabetic lenses which was accompanied by an increase in the level of lipid peroxidation products (LPO) [5].

The surgery has its own limitations; pronounced post-operative inflammation, loss of vitreous humor, posterior capsule opacification and expensive. [6] so there is a need to look at the impact of treating cataract and relate it not just to surgery but also to scholastic achievements and development. In the recent years, there has been explosion of interest in the polymer based delivery systems [7]. By utilization of the principles of sustained release with semi synthetic polymers as embodied by eye drop and ophthalmic gel offers an attractive approach to the problem of prolonging precorneal drug residence times [7,8,9]. The use of gel as a delivery system can increase the residence time of drugs in ocular *cul-de-sac* and consequently enhance bioavailability. Gel delivery systems have several advantages such as the ease of administration, none greasy, patient compliance, high residence time in eye and better drug release [10]. In recent times, herbal drugs and natural products are targeted to develop more safe, effective and economical treatment for prevention or delay the cataract [1,11].

Boerhaavia Diffusa is a novel, multiple action anti-oxidant anti-inflammatory drugs that is currently approved in many countries for the treatment of ophthalmic disease. *Boerhaavia Diffusa* is efficacious in the treatment of cataract due to its phyto constituents.

Therefore, an attempt made to revive our ancient knowledge by developing modern dosage forms and evaluating their therapeutic efficacy in various eye disorders. In the present study eye drop and *in-situ* gel of *aqueous distillate of Punarnava* (*Boerhaavia Diffusa*) have been used and evaluate for efficacy in cataract against sodium selenite induced cataract using Eyetone eye drop (Deys pharmaceutical PVT LTD, India) as standard in male wister rat eyes [12,13,14].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

For the present study well identified samples of plant *Boerhaavia Diffusa* Linn. (Roots) were

collected from their natural habitats along with the samples from local market. The samples have been authenticated by *National Botanical Research Institute,* Lucknow. (NBRI) India (SPECIFICATION No: NBRI-SOP-202).

These were dried and prepared to powdered using laboratory Hammer mill. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight, labeled and stored for distillation [1,8,15].

2.2 Method of Preparation for Arka

Powdered drug (roots of *Boerhaavia diffusa*) was soaked with small amount of water and kept over-night. This makes the drug soft and when boiled releases the essential volatile principles easily. The following morning it is poured into the *Arkayantra* (distillation assembly) and the water in the ratio of (1:16) was added and boiled. The vapor was collected in a receiver. In the beginning, the vapour consists of only steam and may not contain the essential chemical constituents of the drugs and was discarded. The last portion also may not contain therapeutically essential substance and was discarded. The aliquots collected in between contain the active ingredients and is called *Arka* (aqueous distillate) of the drug Punarnava (*Boerhaavia diffusa*) [8].

2.3 Eye Drop of *Boerhavia diffusa*

2.3.1 Method of preparation

The above *Arka* was used for the preparation of eye drop. All the ingredients as given in Table 1 were mixed slowly and volume was made up to 10 ml with aqueous distillate, sterilized by autoclaving followed by U.V. radiation for 30 min. and stored in 10 ml previously sterilized plastic screw capped air tight container. pH was maintained 7.2 continuously by phosphate buffer during preparation [16,17] (Refer Table 1).

2.4 *In-situ* Gel of *Boerhavia diffusa*

2.4.1 Method of preparation

2.4.1.1 By polymer hydration method

According to Table: 2 polymers were taken in a beaker and 2ml (aqueous distillate) was added to it. This was allowed to soak for about 1 hr. After some time (1:16 ml) of *Boerhaavia diffusa* root (aqueous distillate) with other additives was added and volume was made up 100 ml by remaining (aqueous distillate). The stirring was continued to form a homogenous dispersion of the drug in the gel. The gels were buffered with phosphate buffer at a pH of 7.2 to 0.05 and these were sterilized by autoclave / U.V. radiation for 30 min. These were aseptically filled in sterile plastic containers and labeled.

Table 1. Composition of eye drop

Ingredients	Formulation quantity
Punarnava root (gm.)	6.23
Sodium alginate (gm)	0.2
NaCl (gm)	0.09
Benzalkonium chloride (%)	0.01
Aqueous distillate (q.s.)	100.00

Table 2. Compositions of *in-situ* ophthalmic gels

Ingredients	HPMC : SA gel (g)				HPMC : Carbopol gel (g)			
	H1	H2	H3	H4	C1	C2	C3	C4
Punarnava root (gm)	6.23	6.23	6.23	6.23	6.23	6.23	6.23	6.23
HPMC	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2
Sodium alginate/ carbopol	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
Citric acid	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407
Benzalkonium chloride (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Disodium hydrogen orthophosphate	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125
Aqueous distillate (q.s.)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Where: (HPMC: SA gel) is denoted for gel formulated by polymer Hydroxy propyl methyl cellulose and Sodium alginate. And HPMC: Carbopol gel denoted for gel formulated by Hydroxy propyl methyl cellulose and Carbopol by Arka of Punarnava roots

2.4.1.1.1 Animals

The animals in the current study were treated in accordance with the institutional guidelines after approval of Institutional Animal Ethics Committee (IAEC) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules New Delhi. [9] Wister Albino male rats used for the study were obtained from the animal house stock of the Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Moradabad, Uttar Pradesh, India [10]. The rats were housed in large spacious cages, and given food and water *ad libitum*. The animal room was well ventilated and had a regular 12:12-h light/dark cycle throughout the experimental period [11]. For study, 18 Albino male wistar rats of weighing 108-129 g were divided into control and treated groups, Local irritation test was performed to provide estimation of rats ocular response to the tested formulations, after that the efficacies of both formulations (eye drop and gel) were assessed using subcutaneously 0.01% w/v sodium selenite-induced cataract in male wister rats [12]. Measurement of opacification done on wister rats with the help of picture readings of test eye drop and ophthalmic gel [13]. The opacities are determined for 30 days after induction of cataract in rats [14,18].

2.4.1.1.2 Experimental design (Taguchi method) [19]

According to several engineering and pharmaceutical scientists, taguchi method is a designer tool to predict the optimized formulations by considering the results from experimental formulations. The results obtained by taguchi method is mainly depends on many factors (variables) and levels.

Taguchi method detects the optimized formulations by using the tool that is orthogonal array (OA). (OA) is the matrix of numbers arranged in columns and rows, the taguchi method quantify the present variations by signal to noise (S/N) ratio. These (S/N) ratios are used to measure the effect of factors (variables) on performing experimental formulations. According to closeness of the average response of optimized formulations, the (S/N) ratio determines the result from experimental formulations as type of characteristics: smaller is better, nominal is best and larger is better.

This design is 2^2 Taguchi orthogonal array, require four experimental formulations for *in-situ* gel with two parameters (concentration of polymer A (HPMC with Sodium alginate) and concentration of polymer B (HPMC with Carbopol 940), at two levels (high and low) of each. interactions were neglected**.** There are two S/N ratios of common interest for optimization of Static Problems;

(I) Smaller-The-Better:

$n = -10 \text{ Log}_{10}$ [mean of sum of squares of measured data]

This is usually the chosen S/N ratio for all factors (variables) on performing formulations, like "concentration of polymers" etc. for which the ideal value is zero. The generic form of S/N ratio then becomes,

$n = -10 \text{ Log}_{10}$ [mean of sum of squares of {measured - ideal}]

(II) Larger-The-Better:

$n = -10 \text{ Log}_{10}$ [mean of sum squares of reciprocal of measured data]

This case has been converted to Smaller-The-Better by taking the reciprocals of measured data and then taking the S/N ratio as in the smaller-the-better case.

The results obtained by taguchi method is mainly depends on many factors (variables) and levels. Taguchi method detects the optimized formulations by using the tool that is orthogonal array (OA). The best optimized batch for both ophthalmic gel formulations was obtained by analyzing factors (variables) and levels for viscosity, drug content and cumulative drug release as calculated in Tables 1 - 13 and Figs. 1 - 6.

Here 2^2 taguchi designs is applied where 2 levels that is high and low with 2 factors that is concentration of polymers (A) HPMC with Sodium alginate and concentration of polymers (B) HPMC with Carbopol 940 (Tables 1 and 2).

The method is applied on parameters (Viscosity, drug content and percent cumulative drug release), each value is taken in triplicate.

Observation is calculated as mean, standard deviation, log standard deviation and S/N ratio.

S/N ratio is calculated as by formula:

1/ value x =M,+1/2^{nd} value x third value MRC Km+------gives new value---Take log of new

value and multiply with 10--------Final S/N value is determined for individual parameters (Tables 2-9).

A1, A2 and B1, B2 graph for parameters (Viscosity, drug content and percent cumulative drug release) is obtained by taking values by Mean, log standard deviation and S/N ratio for both Gels.

Where A1 is the average of = lower value +lower value /2

Where:

A1 is the average of = lower value + lower value/2,
A2 is the average of = higher value+ higher value/2
B1 is the average of = highest + second highest value/2
B2 is the average of = highest + lowest value/ 2. (Graph Figs. 1-6)

Final A and B was calculated after seeing the graph of (A1, A2 and B1, B2)

A1 is considered as 1 and A2 is considered as 2. B1 is also considered as 1 and B2 is 2.

Then optimized batch was calculated by counting A and B for each parameter of both gels (Table 10-13).

2.4.1.2 Formulation design for in-situ gel

- According to L-4 array, the preparation is designed as 2^2 levels and factors.
- Variables: Concentration of Polymers. Gel (A) HPMC, Sodium Alginate, Gel (B) HPMC, Carbopol 940.
- Levels: Low and High.

Table 3. Level of process parameters

Variable	Levels	
	Low	High
Concentration of polymers	1	1
	1	2
	2	1
	2	2

Table 4. Taguchi L4 orthogonal array for HPMC with sodium alginate ophthalmic gel

No. of formulation	Variables	
	HPMC	Sodium alginate
1	-1	-1
2	-1	+1
3	+1	-1
4	+1	+1

Table 5. Taguchi L4 orthogonal array for HPMC with carbopol 940 ophthalmic gel

No. of formulation	Variables	
	HPMC	Sodium alginate
1	-1	-1
2	-1	+1
3	+1	-1
4	+1	+1

Table 6. Showing concentration of polymers for both ophthalmic gels

S. no.	Variables for HPMC, Sodium Alginate gel at low and high level		Variables for HPMC, Carbopol 940 gel at low and high level	
	Concentration of HPMC	Concentration of Sodium Alginate	Concentration of HPMC	Concentration of Carbopol 940
1	1 (0.001 mg / ml)	1 (0.001 mg / ml)	1 (0.001 mg / ml)	1 (0.001 mg / ml)
2	1 (0.001 mg / ml)	2 (0.002 mg / ml)	1 (0.001 mg / ml)	2 (0.002 mg / ml)
3	2 (0.002 mg / ml)	1 (0.001 mg / ml)	2 (0.002 mg / ml)	1 (0.001 mg / ml)
4	2 (0.002 mg / ml)	2 (0.002 mg / ml)	2 (0.002 mg / ml)	2 (0.002 mg / ml)

Table 7. Experimental data (optimization of viscosity) for HPMC with Sodium Alginate Ophthalmic Gel

S.N	Observed viscosity			Mean	S.D	Log of S.D	S/N ratio
1	1.0	1.3	1.5	2.6	1.5	3.1	28.89
2	4.5	4.7	5.0	3.8	2.2	3.3	30.24
3	3.3	3.8	3.7	3.3	1.9	3.2	59.88
4	2.1	2.9	2.4	3.0	1.7	3.2	57.44

S.D: Standard deviation

Table 8. Experimental data: (optimization of drug content) for HPMC with Sodium Alginate ophthalmic gel

S.N	Observed drug content			Mean	S.D	Log of S.D	S/N ratio
1	90.20	94.80	95.60	93.53	53.99	1.732	60.03
2	95.18	92.12	94.31	93.87	54.19	1.733	0.1463
3	81.17	92.68	84.37	86.07	49.69	1.696	-0.3495
4	89.70	93.30	92.24	91.74	52.96	1.723	29.95

S.D: Standard deviation

Table 9. Experimental data: (optimization of cumulative drug release) for HPMC with Sodium Alginate ophthalmic gel

S.N	Observed cumulative drug release			Mean	S.D	Log of S.D	S/N ratio
1	75.60	75.52	73.55	74.89	43.23	1.63	-0.0562
2	84.87	79.91	80.02	81.60	47.11	1.67	30.006
3	75.83	75.91	76.04	75.92	43.83	1.641	60.007
4	79.91	74.75	69.04	74.56	43.04	1.633	29.65

S.D: Standard deviation

Table 10. Experimental data (optimization of viscosity) for HPMC with Carbopol 940 ophthalmic gel

S.N	Observed viscosity			Mean	S.D	Log of S.D	S/N ratio
1	2.8	2.9	2.9	2.86	1.6	3.22	30.10
2	2.9	2.8	2.0	2.56	1.5	3.17	88.46
3	3.0	3.6	3.8	3.4	2.0	3.30	90.24
4	2.5	3.1	3.0	2.8	1.6	3.22	59.94

S.D: Standard deviation

Table 11. Experimental data: (optimization of drug content) for HPMC with Carbopol 940 ophthalmic gel

S.N	Observed drug content			Mean	S.D	Log of S.D	S/N ratio
1	92.4	94.4	87.9	91.5	52.82	1.722	-0.2596
2	93.70	91.2	92.1	92.33	53.30	1.726	30.04
3	93.6	93.8	94.2	93.86	54.19	1.733	0.064
4	90.1	93.5	91.8	91.8	53.00	1.724	40.82

S.D: Standard deviation

Table 12. Experimental data: (optimization of cumulative drug release) for HPMC with Carbopol 940 ophthalmic gel

S.N	Observed cumulative drug release			Mean	S.D	Log of S.D	S/N ratio
1	74.06	75.15	73.72	74.31	42.90	1.632	89.91
2	79.60	81.52	73.49	80.17	46.28	1.665	89.54
3	85.71	85.00	86.81	85.84	49.55	1.695	60.09
4	78.61	75.58	75.98	76.72	44.29	1.646	77.53

S.D: Standard deviation

2.4.1.3 Evaluation of eye drop and gel

2.4.1.3.1 Determination of pH

Accurately 2.5 ml of eye drop and gel was weighed and dispersed in 25 ml of distilled water. The distilled water was used because it has aqueous solubility and it also match with the pH of lachrymal fluid. The pH of the eye drop and gel was measured using glass electrode pH meter. [20].

2.4.1.3.2 Determination of viscosity

Viscosity of the gel was determined using a Brook field Viscometer, Spindle No 2 (Brookfield Engineering Labs., USA). All the formulated gels were sheared at 1.6-7.4 torque for 5 min. The

shear stress was recorded for each formulation [21].

Table 13. Summary of analyses of factor effects for HPMC with Sodium Alginate ophthalmic gel

Factor	Mean	Log(s)	S/N ratio
Viscosity			
A	+2	+2	+2
B	+1	+1	+1
Drug content			
A	+2	+2	+2
B	+1	+1	+1
***In vitro* cumulative drug release**			
A	+2	+2	+2
B	+1	0	+1

Table 14. Summary of analyses of factor effects for HPMC with carbopol 940 ophthalmic gel

Factor	Mean	Log(s)	S/N ratio
Viscosity			
A	+2	0	+2
B	+1	+1	+1
Drug content			
A	+2	+2	+2
B	+1	+1	+2
***In vitro* cumulative drug release**			
A	+2	+2	+2
B	+1	+1	+1

Table 15. Final optimized parameters values for HPMC with Sodium Alginate ophthalmic gel

Factor	Optimized level
A	+1 (High conc. Of HPMC)
B	-1 (Low conc. Of Sodium alginate)

Table 16. Final optimized parameters values for HPMC with Carbopol 940 ophthalmic gel

Factor	Optimized level
A	+1 (High conc. Of HPMC
B	-1 (Low conc. Of Carbopol 940)

2.4.1.3.3 Clarity testing (IP 2007)

Clarity test was done against dark and white background board apparatus, for the presence of foreign particles [22].

2.4.1.3.4 Phytochemical analysis

Phytochemical analysis of Arka, eye drop and *in-situ* gel was carried out by different methods [23] (refer Table 18).

2.4.1.3.5 Determination of mucoadhesives strength

Mucoadhesives strength of 1% aqueous solution of optimized batch (H3 and C3) was studied with QTS-25 Texture Analyzer (Brookfield Engineering Labs., USA). Freshly excised goat by using goat conjunctival mucosa, was attached to the upper probe of the instrument, and drop of 1% gel solution was kept below that. The upper probe was then lowered at a speed of 10 mm/min to touch the surface of the solution. A force of 100g was applied for 25 s, respectively, to ensure intimate contact between the membrane and the gel. The surface area of exposed mucous membrane was 1.13 cm2 (Shyamoshree and Bandyopadhyay, 2010). The studies were conducted for HPMC (15) cps, sodium alginate and Carbopol 940, and results were compared [24].

2.4.1.4 Determination of drug content

Drug content was determined by dissolving accurate weighed quantity of eye drop and gel in PBS 7.2. After suitable dilutions the absorbance was recorded by UV Vis spectrophotometer at 273.6 nm. Drug content was determined using slope of the standard curve previously plotted [25].

2.4.1.5 *In-vitro* release study

It was done with 25 mm with Franz diffusion cell (KC) cell by using egg membrane for the study. The membrane was transparent and regenerated cellulose type, which was permeable to low molecular weight substances. The semi permeable membrane was tied on one end of open ended cylinder (diameter 1.6 cm^2) which was act as donar compartment. 10 ml of eye drop / gel was placed inside the compartment the semi permeable membrane acts as corneal epithelium.

The entire surface of the membrane was in contact with the receptor compartment containing 25 ml of isotonic buffer pH 7.2. The receptor compartment was continuously stirred at (50 rpm) using magnetic stirrer. The temperature was maintained 37℃.

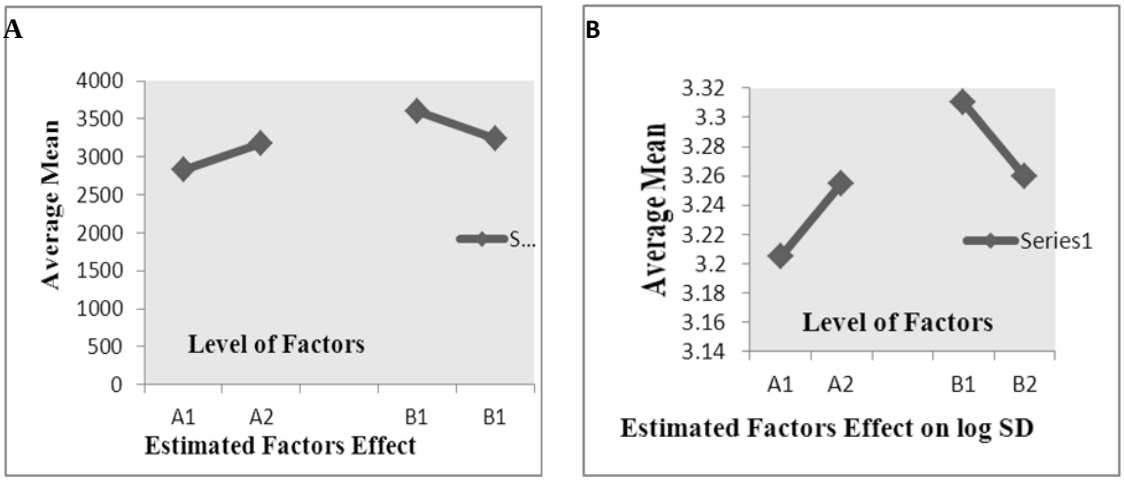

Fig. 1. Viscosity graphs HPMC with Sodium Alginate gel
(A) Estimated factor effect; (B) Estimated factor effect on logs

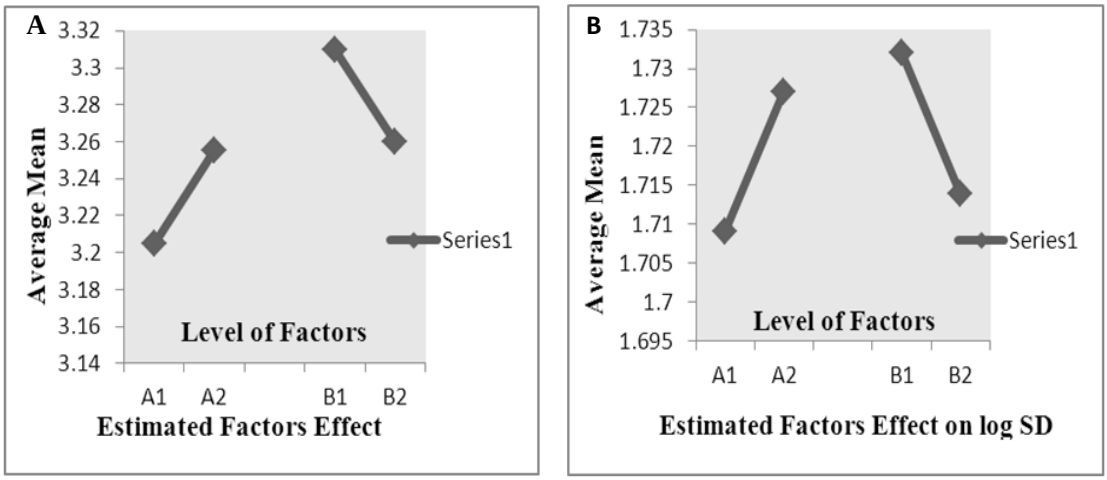

Fig. 2. Drug content graphs for HPMC with Sodium Alginate gel
(A) Estimated factor effect; (B) Estimated factor effect on logs

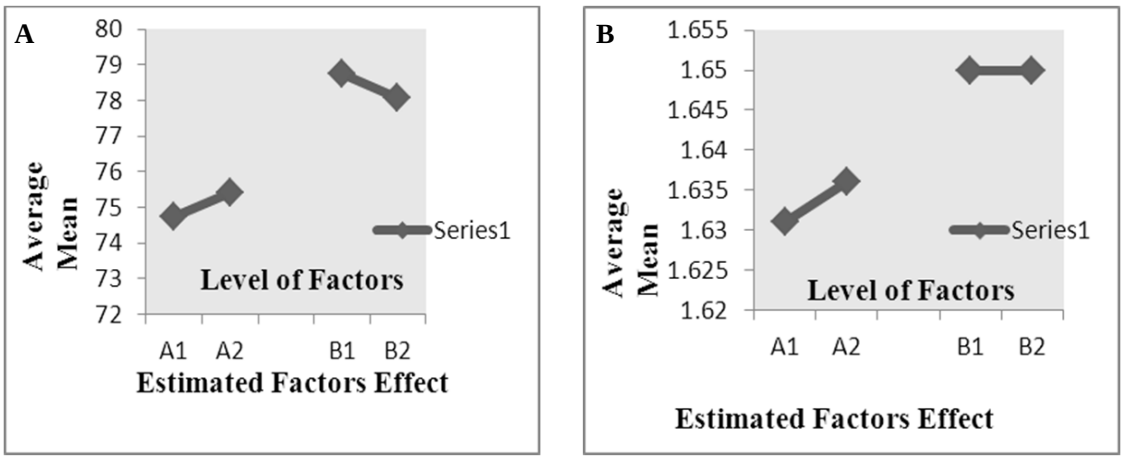

Fig. 3. Cumulative drug release graphs HPMC with Sodium Alginate gel
(A) Estimated factor effect; (B) Estimated factor effect on logs.

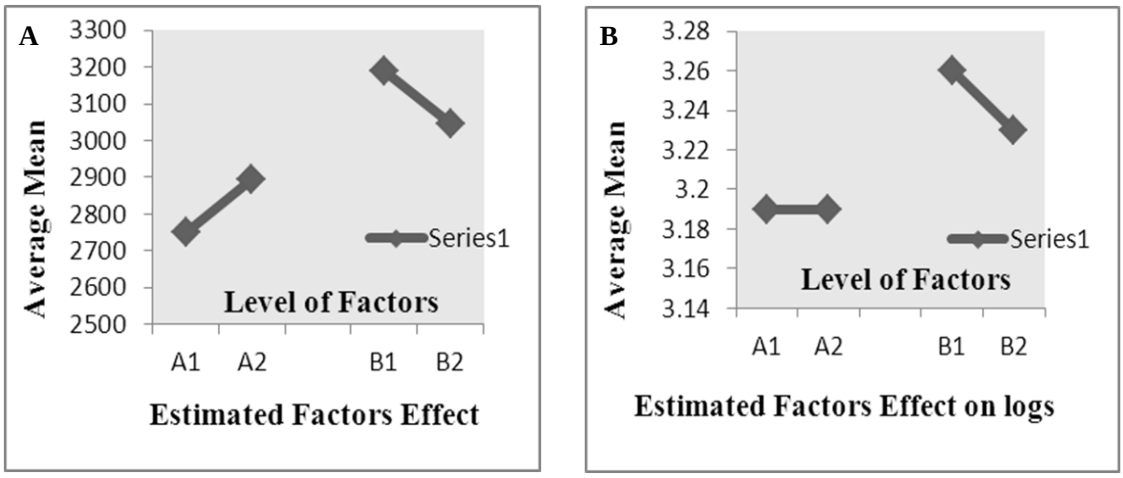

Fig. 4. Viscosity HPMC with Carbopol 940
(A) Estimated factor effect; (B) Estimated factor effect on logs.

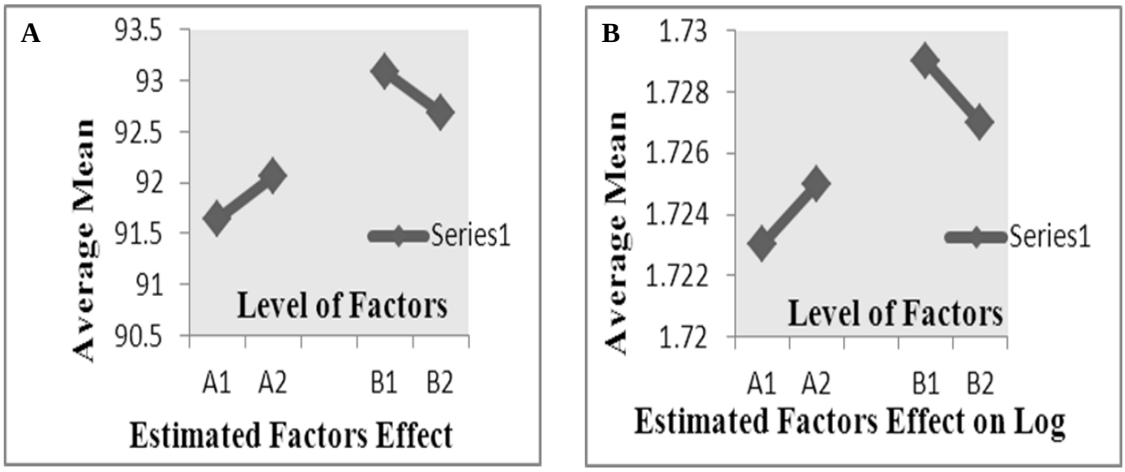

Fig. 5. Drug Content HPMC with Carbopol 940 gel
(A) Estimated factor effect; (B) Estimated factor effect on logs

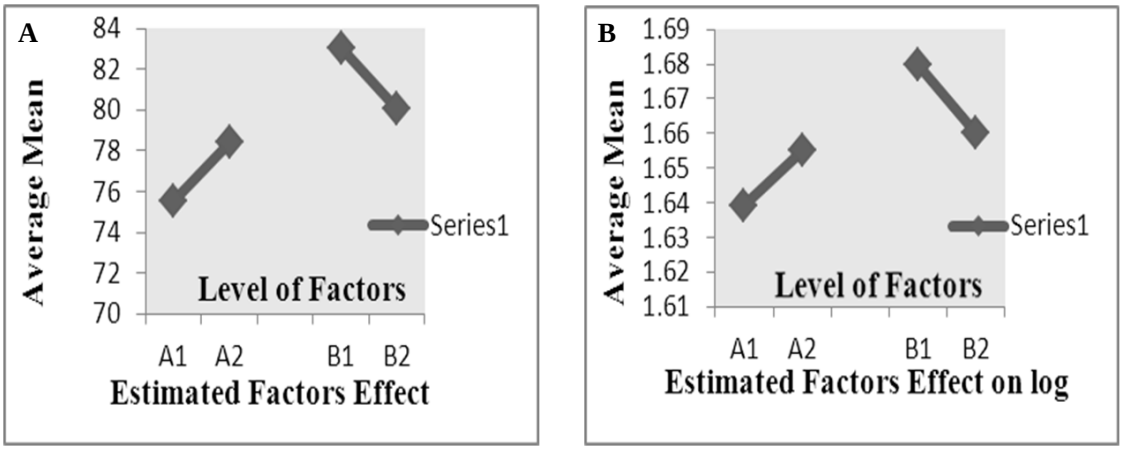

Fig. 6. Cumulative drug release graphs HPMC with Carbopol gel.
(A) Estimated factor effect; (B) Estimated factor effect on logs

The study was carried out for 9 hr. The sample was withdrawn at predetermined time intervals and the same volume was replaced with fresh buffer medium. The absorbance of the withdrawn sample was measured after suitable dilutions at 273.6 nm to estimate the drug. The experiment was carried out in triplicate and average values were reported [26].

2.4.1.6 Drug release kinetics

The drug release data was plotted using various kinetics, such as, zero order and first order. Higuchi's kinetics and Korsmeyer's equation were used to evaluate the drug release mechanism. The obtained data of present study for zero order equation were studied from *in vitro* drug release and were plotted as cumulative amount of drug release versus time (Jain, 2007). C = K0t [27].

2.4.1.7 Stability study

Chemical and physical stabilities of optimized formulations were assessed under various storage conditions, namely room temperature (RT), 5±1 and 40±1℃ and 75% RH as per ICH guideline [28].

2.4.1.8 *In-vivo* studies

2.4.1.8.1 Ocular irritation test

The potential ocular irritancy effects of the formulations were evaluated by observing for any redness, inflammation and increase in tear production. Local irritation test was performed to provide estimation of rat's ocular response to the tested products. Both formulations (eye drop and *in-situ* gel) were tested on male wister rats. The treatment was performed by two drop instillation of eye drop and gel (0.62gm./10ml)under tests into the conjunctival sac of left eye every 12-12 hrs. for 3 days. Plain placebo formulations were instilled into the right eye. Both eyes of the rats under test were examined for any signs of irritation before treatment and up to 10 h after instillation. [29,30] observed eye irritation of the cornea, iris, conjunctiva at 1, 2, 3, 4 & 7 day. There wasn't physical problem at 3 rats. There wasn't eye irritation, inflammation and increase in tear production of the cornea, iris, and conjunctiva at 1, 2, 3, 4 & 7 day [31]

2.4.1.9 Efficacy study

To assess cataract, male wister rats were divided into three groups, (n=6), groups-1 Control, groups-2 Ophthalmic drop (eye drop), groups-3 *In-situ* gel [32]. The control group received only distilled water in both eyes, two drops in each eye, two times in a day, and for treatment of disease, In eye drop and *in-situ* gel group, test (Aqueous Distillate of *Boerhavia Diffusa* roots) and standard drug (Itone) Eye drop, manufactured by Deys Pharmaceutical PVT LTD, Calcutta was applied on right and left eyes of same animal [33]. In order to induce lens opacity, sodium selenite 0.01% w/v subcutaneously) was injected to the male Wister rats. Opacity was developed from12-48 hrs. Which were already divided into control (distilled water) group (n= 6) and treatment group of ophthalmic drop and *in-situ* gel group (n= 6) [34]. Then along 30 days(four weeks) post injection of sodium selenite, by using ophthalmoscope grading criteria, the score of lens opacity (cataract maturity) was determined [35].

2.4.1.10 Induction of cataract

Sodium selenite (0.01% w/v subcutaneously) was injected to the rats. Opacity was developed from 12-48 hrs. Rats were already divided into control (distilled water) group (n= 6) and treatment group of ophthalmic drop and *in-situ* gel group (n= 6). Then along 30 days (four weeks) post injection of sodium selenite. By using ophthalmoscope grading criteria, the score of lens opacity (cataract maturity) was determined.

Tropicamide (0.5%) and Phenylephrine (10%) were used to obtain maximum pupillary dilatation during examination [12].

2.4.2 Study design and groups

Rat eyes were divided into three groups each group having six rats.

Group-I: Normal control (given only distilled water in both eyes)

Group-II: Ophthalmic Eye drop (0.03ml/10ml) (right eye of same rat is considered as test and left eye considered as standard).

Group-III: *in-situ* gel (0.03ml/10ml) (right eye of same rat is considered as test and left eye considered as standard *in-situ* gel).

Standard drug: Itone Eye drop [Deys. Kolkata, India: Marketing Division, Dey's Medical Stores (Mfg.) Ltd.] [14].

2.4.3 Morphological and photographic evaluation

Lenses were clearly visible through the eyes and observed to measure lens opacity. The degree of opacity was graded as follows:

0 : Absence
+ : Slight degree
++ : Presence of diffuse opacity
+++ : Presence of moderate diffuse opacity
++++: Presence of extensive thick opacity [12,14].

3. RESULTS AND DISCUSSION

3.1 The pH

The pH of the optimized formulations eye drop was 7.23 and in-situ gel batch H3 was 7.2 and batch C3 was 7.2. So this would not produce any irritation after administration (Optimization according to Taguchi design) (Tables 14 and 16).

3.2 Viscosity

The viscosity of various formulated ophthalmic *in-situ* gels from aqueous distillate of *Boerhaavia diffusa* roots by (HPMC, sodium alginate) and (HPMC, Carbopol 940) is shown in Tables 14 and 16. Viscosities were determined for optimized batch H3 as 3.33 cps and batch C3 as 2.78 cps. This was due to the concentration of polymer. When the concentration of polymers increased, the viscosity may also increase the interaction between polymers form gel (Tables 14 and 16).

3.3 Mucoadhesives Strength

The mucoadhesives strength of optimized formulations provides intimate contact of gel to the ocular *cul-de-sac* and improves sustained action of drug. The mucoadhesives strength was determined by texture analyzer using goat conjunctival mucosa. The applied force by probe was 100 g and residence time was 25 s. As per observations obtained from Table 3, batch H3 (HPMC, sodium alginate gel) and batch C3 (HPMC, Carbopol 940 gel) show H3 (14.49) and C3 (13.83) according to Taguchi design (Tables 14 and 16).

3.4 Drug Content

The drug content values of all batches from both eye drops and ophthalmic gels were found to be in range between 95 and 99% (Tables 14 and 16).

3.5 Clarity Testing

All formulations were found to be transparent and slight turbid, clear. Due absence of foreign particles formulation leads no ocular irritation. So the preparations are free from foreign particle so that it improves ocular tolerance.

Table 17. Physico-chemical parameters of eye drop

Physicochemical parameters	Eye drop formulations			
	E1	E2	E3	E4
pH*	6.9±0.13	7.20±0.252	7.25±0.274	7.23±0.16
Drug Content* (%)	94.8±0.7023	95.1±0.7483	94.7±0.086	95.4±0.2645

Table 18. Physico-chemical parameters of the optimized formulations. HPMC with Sodium Alginate Ophthalmic Gel

Optimized Batch : H3			
pH	Viscosity	Drug content	Mucoadhesive strength
7.2	3.33 cps	95.4	14.49

Table 19. Physico-chemical parameters of the optimized formulations. HPMC with Carbopol 940 Ophthalmic Gel

Optimized Batch : C3			
pH	Viscosity	Drug content	Mucoadhesive strength
7.2	2.78	94.1	13.83

3.6 Phytochemical Analysis

The aqueous distillate so prepared has been tested qualitatively for the presence of different groups of compounds and details are as below [10].

3.7 *In-vitro* Release Study

The results of the *in-vitro* release study from different gels by the KC cell (25 mm) are as shown in Fig. 1. *In vitro* release for eye drop and both gel formulations showed a linear relationship between cumulative percentage releases versus time. In eye drop release rate was 80.56% and H3 batch of HPMC, SA *in-situ* gel showed 84.66% drug release. In C3 batch of HPMC, Carbopol 940 showed 82.47% drug release, through KC cell within a period of 9 h. Drug release obeys zero order kinetics (Fig. 2). The *in vitro* release order of different ophthalmic gel formulations was expressed in the decreasing order after 9 h. The difference in release rate could be due to viscosity and release of aqueous distillate in HPMC with SA and HPMC with Carbopol 940 *in-situ* gel. As given in (Tables 15 and 17 and Figs. 7 and 8).

3.8 Drug Release Kinetics

The release profiles of optimized formulation were best described by a model that represents systems where drug diffusion occurs through a polymeric structure or network, $Mt/M\infty = kt^n$ (r2=0.942) and (0.902) where $Mt/M\infty$ is the fractional release of the drug, t is the release time, k is a constant, and n is the release constant, indicative of the mechanism of drug release. From Table 8, it was clear that optimized formulations and zero order release kinetics E4 (eye drop) have r2= 0.939, H3 (in-situ gels) have r2=0.952 and C3 have r2=0.941 (Tables 20 and 21).

Table 20. Phytochemical tests

Preliminary phytochemical tests for aqueous distillate of roots of *Boerhavia Diffusa* by different solvents for presence of natural constituents.			
S. no	**Natural products**	**Test performed**	**Result (in triplicate)**
1.	Alkaloid	Dragendorff's test	+ ve
2.	Flavone	Alkaline test	+ve
3.	Steroid	Shinoda test	+ve
4.	Tannin	Liebermann- Burchard reagent	-ve
5.	Sugar	Liebermann- Burchard reagent	-ve
6.	Terpenoid	Liebermann- Burchard reagent	+ve
7.	Saponin	Neutral $FeCl_3$	+ve
8.	Glycoside	Molisch's test	+ve
9.	Glycoside	Noller's test	+ve
10.	Glycoside	NaOH solution	+ve
11.	Glycoside	Brontragar's test	+ve

Table 21. *In-vitro* drug release study of various eye drop formulations

Time in hrs	% cumulative drug release ± S.D.			
	Ayurvedic Eye Drop Formulations			
	E1	**E2**	**E3**	**E4**
0	0	0	0	0
0.08	10.38±0.48	12.14±0.51	15.14±0.335	15.81±0.571
0.25	12.91±0.45	17.76±0. 675	19.20±0.424	21.40±0. 2 60
0.50	16.42±0.24	27.18±0. 471	24.50±0.289	29.91±0. 7 79
1	23.19±0.508	30.51±0. 221	28.84±0.455	37.34±0. 282
2	28.71±0.177	36.42±0. 240	33.27±0.181	46.85±0. 25
4	36.59±0.138	42.81±0. 153	45.21±0.192	49.82±0. 170
6	49.49±0.212	53.72±0. 225	59.61±0.275	57.96±0. 100
8	57.51±0.32	69.46±0. 198	71.48±0.235	69.31±0. 176
9	65.83±0.32	78.21±0. 421	76.20±0.560	80.56±0. 439

Table 22. *In vitro* drug release of optimized batch H3

Optimized Batch code H3	Time in hrs									
	0	**0.08**	**0.25**	**0.50**	**1**	**2**	**4**	**6**	**8**	**9**
	0	16.81±0.571	20.40±0. 260	26.91±0. 779	32.54±0. 28 2	36.47±0. 25	49.82±0. 170	61.96±0. 100	79.41±0. 17 6	84.66±0. 439

Table 23. *In vitro* drug release of optimized batch C3

Optimized Batch code C3	Time in hrs									
	0	**0.08**	**0.25**	**0.50**	**1**	**2**	**4**	**6**	**8**	**9**
	0	17.04±0.280	21.32±0.195	25.89±0.096	29.91±0.508	3 4.75±0.091	43.86±0.136	52.62±0.220	67.91±0.136	82.4 7±1.15

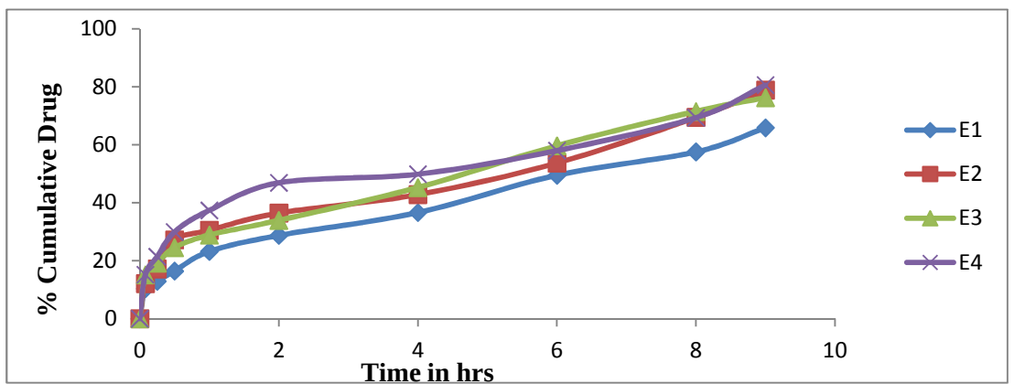

Fig. 7. Cumulative drug release graph of Ayurvedic eye drop, Where E1, E2, E3, E4 are no of formulations

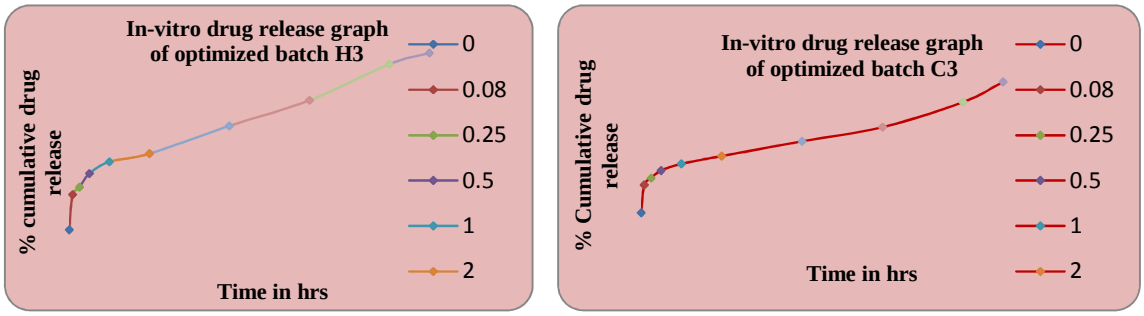

Fig. 8. HPMC with Sodium Alginate gel and HPMC with Carbopol gel In-vitro drug release studies of various formulations

Table 24. Shelf-life of optimized formulation batch H3

S. No.	Parameters	Storage conditions		
		5±1°C	Room temperature	40±1°C, 75%(RH)
1	K (day^{-1})	3.65×10^{-4}	2.5×10^{-4}	5.3×10^{-4}
2	$t_{½}$ (days)	1874.53	2736.84	1290.92
3	$T_{10\%}$ (days)	284.93	416	196.22

Table 25. Shelf-life of optimized formulation batch C3

S. No.	Parameters	Storage conditions		
		5±1°C	Room temperature	40±1°C, 75% (RH)
1	K (day^{-1})	3.5×10^{-4}	3.3×10^{-4}	6.6×10^{-4}
2	$t_{½}$ (days)	1943.75	2073.35	1036.6
3	$T_{10\%}$ (days)	295.45	3315.15	157.57

Table 26. Diffusion kinetics of various formulations of Ayurvedic eye drop

Batch code	Higuchi equation		Korsmeyer's Peppas equation		First order equation		Zero order	
	N	R^2	N	R^2	N	R^2	N	R^2
E1	21.90	0.97	0.361	0.208	0.105	0.454	6.771	0.933
E2	24.99	0.97	0.39	0.239	-0.393	0.050	7.738	0.937
E3	22.79	0.97	0.391	0.239	0.105	0.44	7.040	0.939
E4	24.24	0.97	0.404	0.250	0.108	0.461	7.523	0.939

N- Release exponent, R- Correlation coefficient

Table 27. Diffusion kinetics parameters of optimized formulation H3

Batch code	Higuchi equation		Korsmeyer's Peppas equation		First order equation		Zero order	
	N	R^2	N	R^2	N	R^2	N	R^2
H3	24.24	0.97	0.404	0.250	0.108	0.461	7.523	0.952

N- Release exponent, R- Correlation coefficient

Table 28. Diffusion kinetics parameters of optimized formulation C3

Batch code	Higuchi equation		Korsmeyer's Peppas equation		First order equation		Zero order	
	N	R^2	N	R^2	N	R^2	N	R^2
C3	17.57	0.934	0.321	0.207	0.097	0.432	5.437	0.941

N- Release exponent, R- Correlation coefficient

3.9 Stability Studies

Stability studies of optimized formulations were carried out on the basis of ICH guidelines and the observed values of K (Stability constant), t½ (half-life), and T10% (shelf-life) of optimized formulation after different storage conditions are shown in Tables 18 and 19.

***In-vivo* studies**

Registration No: 837/ac/04/CPCSEA
Resolution No: 2014/837ac/PGD/01

The animal ethical committee, Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Moradabad, Uttar Pradesh, India) had given permission for the *in vivo* study.

3.10 Ocular Irritation Test

The potential ocular irritancy effects of the formulations were evaluated by observing redness, inflammation or increase in tear production. Local irritation test was performed for rat's ocular response to the tested formulations. The test was performed on 3 rats by instilling eye drop, gel (0.62 gm./10 ml) and standard Itone Eye drop (0.5 gm./10 ml) [Deys. Kolkata, India: Marketing Division, Dey's Medical Stores (Mfg.) Ltd.] to the left eye only, The animals 1-3 received 2 drops each of eye drop, gel and standard every 12-12 hrs in a day for three days. Placebo plain formulation was used to the right eye of 1-3 rats respectively. Both eyes of the rat under test were examined for any signs of irritation before treatment and up to 10 h after instillation. In the measurement of injury to the eye, a modification of the scoring system of Friedenwald, Hughes and Herrmann (Modified Draize Technique) was used. And readings were made at 1, 24 and 48 hours after instillation of the formulation into the eye and were evaluated [36].

3.11 Efficacy Study

To assess cataract, male wister rats were divided into three groups, (n=6), groups-1 Control, groups-2 Ophthalmic drop (eye drop), groups-3 *In-situ* gel [37]. The control group received only distilled water in both eyes, two drops in each eye, three times in a day, and for treatment of disease eye drop and *in-situ* gel group was applied on right eyes of group-2 and group-3rd and standard drug (Itone) Eye drop, manufactured by Deys Pharmaceutical PVT LTD, Calcutta was instilled into left eyes of group-2 and group-3rd of same wister rats. In order to induce lens opacity, sodium selenite 0.01% w/v subcutaneously) was injected to the male Wister rats. Opacity was developed from 12-48 hrs. Which were already divided into control (distilled water) group (n= 6) and treatment group of ophthalmic drop and *in-situ* gel group (n= 6).Then along 30 days (four weeks) post injection of sodium selenite, by using ophthalmoscope grading criteria, the score of lens opacity (cataract maturity) was determined.

3.12 Induction of Cataract [14]

Sodium selenite (0.01% w/v subcutaneously) was injected to the male wister rats. Opacity was developed from 12-48 hrs. Rats were already divided into control (distilled water) group (n= 6) and treatment group of ophthalmic drop and *in-situ* gel group (n= 6). Then along 30 days (four weeks) post injection of sodium selenite. By using ophthalmoscope grading criteria, the score of lens opacity (cataract maturity) was determined.

Tropicamide (0.5%) and Phenylephrine (10%) were used to obtain maximum pupillary dilatation during examination [12].

3.13 Study Design and Groups

Rat eyes were divided into three groups each group having six rats.

Group-I: Normal control (given only distilled water in both eyes)

Group-II: Ophthalmic Eye drop (0.62gm./10ml) (right eye of same rat is considered as test and left eye considered as standard).

Group-III: *in-situ* gel (0.62gm./10ml) (right eye of same rat is considered as test and left eye considered as standard *in-situ* gel).

Standard: Itone Eye drop (0.5gm./10 ml) [Deys. Kolkata, India: Marketing Division, Dey's Medical Stores (Mfg.) Ltd.] [14].

3.14 Morphological and Photographic Evaluation

Lenses were clearly visible through the eyes and observed to measure lens opacity [38]. The degree of opacity was graded as follows:

0 : Absence
+ : Slight degree
++ : Presence of diffuse opacity
+++ : Presence of moderate diffuse opacity
++++ : Presence of extensive thick opacity [12,14]

3.15 Result

3.15.1 *In-vivo* studies

3.15.1.1 Ocular irritation test

In-vivo ocular irritation was carried out using rats and as per Draize tests protocol as Table 29.

According to Table 29 none of the optimized formulation showed any sign of redness, inflammation and increase in tear production, after comparison with placebo formulations [33,34,39].

3.15.2 *In-vivo*

3.15.2.1 Lens morphology / photographic evaluation

All six Wister male rats (108-129 gms range) in group I (distilled water) remained normal. And in group II (eye drop) all six lenses showed slight any opacity (Fig. 9). The opacity progressively increased towards the center with complete opacification by 48 hours. Eye drop at 0.62 gm / 10 ml of aqueous distillate, decrease the development of opacity. Compared to group II (eye drop) group III (ophthalmic gel) affectively retarded the development of opacity. The grades of opacity were 0, + and 0 in group I, II and III respectively. The ophthalmic gel showed best result as compared to eye drop by picture readings. Shows equal delay of cataract development as standard (Fig. 9).

The opacities are determined for 30 days after induction of cataract in rats of 30 days' time.

Images of cataract formation, utilizing a slit-lamp microscope at 10 × original magnifications and a digital camera in macro mode are shown.

Group-I: Normal control (given only distilled water in both eyes)

Group-II: Ophthalmic Eye drop (0.62gm./10ml) (right eye of same rat is considered as test and left eye considered as standard).

Group-III: *In-situ* gel (0.62gm. /10ml) (right eye of same rat is considered as test and left eye considered as standard *in-situ* gel).

Standard: Itone Eye drop (0.5gm. /10ml) contain Punarnava with other constituents [Deys. Kolkata, India: Marketing Division, Dey's Medical Stores (Mfg.) Ltd.].

Fig. 9.1. Instillation of eye drop

Fig. 9.2. Instillation of in-situ gel

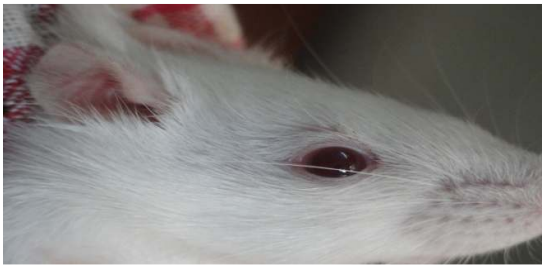

Fig. 9.3. Normal eye after 3 days of eye drop Instillation

Fig. 9.4. Normal eye after 3 days of *in-situ* gel Instillation

Fig. 9. Ocular irritation test of Formulations

Group I:

Before treatment **After treatment**

Group II:

Before treatment **After treatment** **Standard (after treatment)**

Group III:

Before treatment **After treatment** **Standard after treatment**

Fig. 10. *In-vivo* study of Formulations

Table 29. Rats eye irritation test

Redness/ inflammation, tear production	Normal rating	Rating for formulations		
		Eye drop	*Gel*	Standard
Vessels normal/Iris	0 none	0	0	0
Vessels definitely injected above Normal/ Iris folds above normal, congestion, swelling, iris reacts to light	1 slight	0	0	0
More diffuse, deeper crimson red with Individual vessels not easily dissemble	2 moderate	0	0	0
Diffuse beefy red, no reaction to light, haemorrhage, gross destruction	3 Severe	0	0	0

3.16 Discussion

The phytochemical analysis through TLC, HPTLC, GCMS and HPLC of formulations in the form of ophthalmic drop and *in-situ* gel using beta sitosterol as marker component of *arka Boerhaavia diffusa* revealed the presence of valuable terpenoids and sterols. They are very potent in small amount and have a broad range of biological activities, (such as cataract, glaucoma etc) It believed that beta sitosterol detected 20.03% in GCMS analysis have lipophilic action and showing best antioxidant property for the treatment of cataract which increase the efficacy of formulations of *Boerhaavia diffusa* roots.

4. CONCLUSIONS

Boerhaavia diffusa root's ophthalmic drop and *in-situ* gel exerted following results:

- ✓ Affirmative detectable preventive effect against sodium selenite-induced cataract in Wister rats.
- ✓ In comparison to Standard drug (Itone), ophthalmic drop, *in-situ* gel delay the equivalent progression of cataract solicits best result.
- ✓ *In-situ* gel showed best result over ophthalmic drop.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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