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Research Article

PREPARATION OF OCULAR IN SITU GEL FOR GLAUCOMA TREATMENT USING ISOLATED FORSKOLIN FROM *COLEUS FORSKOLII* ROOT

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ABSTRACT

The loss of drug eye environment by various routes is overcome by formulating the viscous formulations. The aim of this ingestion was to optimize and anti glaucoma activity evaluation of in situ gel containing extracted forskolin. Various polymers are used and investigated the effect and optimized the formulation on the bases of In Vitro gelation and in vitro drug release. Temperature, pH and ion activated polymers are used to optimize the formulations and were evaluated for drug content, clarity, and gelling capacity. In vivo intra ocular pressure (IOP) reduction studies and results observed in acceptable range. Based on gelation time (sec) and in vitro percentage drug release P8 formulation was found to be best optimized formulation from the developed by 23 factorial design. This study revealing that the forskolin effectively reduced the glaucoma in rabbits.

Keywords: Two-level factorial design, Forskolin, In vivo intra ocular pressure reduction, In Vitro gelation time.

INTRODUCTION

Coleus forskolii grows wild in arid and semi-arid regions of India, Nepal and Thailand; the roots have long been used in Ayurvedic medicine1. A member of the mint family, it has been traditionally used to treat heart and lung disease, intestinal spasms, insomnia, and convulsions[1]. A labdane diterpenoid, is considered the active secondary metabolite because of its ability to activate the enzyme adenylate cyclase[2]. Recent research has shown that forskolin has positive effects against a wide range of conditions such as asthma, glaucoma, hypertension, hair loss, Cancer and obesity [3,4,5]. The extent of absorption of an ophthalmic drug is severely limited by physiological contraints. Among the factors that limit ocular absorption is the relatively impermeable corneal barrier. The cornea consists of three membranes, the epithelium, the endothelium and inner stroma, which are the main absorptive barriers. The epithelium facing the tears with lipophilic cellular layers, acts as a barrier to ion transport. The tight junctions of the corneal epithelium serve as a selective barrier for small molecules and they prevent the diffusion of macromolecules via the para cellular route. The stroma beneath the epithelium is a

highly hydrophilic layer making up 90% of the cornea. The corneal endothelium is responsible for maintaining normal corneal hydration[6].

The present investigation is aimed to prepare forskolin in situ gels and was evaluated for anti glaucoma activity.

MATERIAL AND METHODS:

Coleus forskolii root procured from department of botany, sri Venkateswara University. Polaxomer F68, Carbopol 940, Sodium Alginate were gifted by Dr. reddy's laboratories, Hyderabad. All the solvents were of High Performance Liquid Chromatography (HPLC) grade. Triple-distilled water was used throughout the studies.

Extraction of Forskolin:

The *Coleus forskolii* root was collected washed and dried. After drying the root was pulverized into granules. By using methods whole forskolii extracted in crude from using methanol as solvent. Methanol extract is concentrated and chloroform is added to concentrate and equal volume of water is added to the separating funnel and shaked well. Allow to settle and separate the chloroform layer. Repeat the water treatment two to three times and collect chloroform layer. Concentrate the chloroform layer. Precipitate the Forskolin (FSK) using ice cold n-hexane. A reddish brown to brown colour powder of Forskolin is obtained. The purification was done by treating the obtained FSK with ether and methanol solvent mixture and vacuum was applied to remove the solvent and lyophilized.

Preparation of in situ gel with purified FSK:

FSK is slightly soluble in water for this reson the solid dispersion was prepared using beta cyclodextrin by kneading method. Accurately weighed quantity of Polaxomer F68 was dispersed in 50ml of purified water; Carbopol 940 and Sodium alginate were sprinkled over this solution, stirred with an overhead stirrer and allowed to hydrate overnight at room temperature. FSK solid dispersion (equivalent to 50 mg of active ingredient) was dissolved in small quantity of acidic medium (2% of HCl solution), 0.03% v/v of benzalkonium chloride (BKC) was added and pH was adjusted to 6.0 by using 0.1N sodium hydroxide solution. The drug solution was added to the polymer solution and stirred homogeneously using magnetic stirrer, losotonicity was adjusted by addition of 0.9% w/v sodium chloride (NaCl) solution. Purified water was then added to make up the volume to 100ml and the solution was filtered through 0.2µm membrane filter and all formulations were sterilized in an autoclave at 121°C for 20 min 7.

Optimization by 23 factorial design :

A three-factor, two-level factorial design (23) were employed for optimization procedure with quantity of Carbopol 940 (B), Sodium alginate (C), and Polaxomer F68 (a) as three prime selected independent variables, which were varied at two levels, low level (−1) and high level (+1). The values of two coded levels of three factors were assumed after preliminary trials. The In vitro gelation time (sec) and In vitro % drug release were measured as dependent variables. Design-Expert® 9.0.3 Software was used for the generation and evaluation of the statistical experimental design. The factorial designed batches and responses are shown in (Table 1).

Table 1: Composition of different coded values in 23 full factorial design

Formulation	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2
		A: Polaxomer F68 mg	B: Carbopol 940 mg	C: Sod. Alginate mg	In Vitro gelation Sec	In Vitro % drug release %
P6	1	2.5	150	100	92	51.7±1
P5	2	2.5	100	100	80	76.7±2
P7	3	2.5	100	150	100	62.2±2
P8	4	2.5	150	150	125	48.12±2
P3	5	2	100	150	100	71.12±2
P4	6	2	150	150	105	52±3
P1	7	2	100	100	82	80.1±2
P2	8	2	150	100	96	66.84±2

Factor	Name	Units	Minimum	Maximum	Coded Values	Mean	Std. Dev.
A	Polaxomer F68	mg	2	2.5	-1.000=2; 1.000=2.5	2.25	0.267261
B	Carbopol 940	mg	100	150	-1.000=100;1.000=150	125	26.7261
C	Sod. Alginate	mg	100	150	-1.000=100;1.000=150	125	26.7261

pH:

pH of the In-situ gels after addition of all ingredients was measured using digital pH meter[8].

Gelation studies:

Gelation studies were carried out in test tube, The studies were carried out using simulated tear fluid (STF) of composition 1 (sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g and purified water sufficient to make 100 g) and of composition 2 (bovin serum albumin 0.268 g, lysozyme 0.268 g, γ-globulin 0.134 g, calcium chloride dehydrate 0.008 g, D-glucose 0.15 g, sodium chloride 0.65 g and distilled water sufficient to make 100 g), which simulated either the divalent cation content or both the protein and divalent cation content of the tear fluid equilibrated at 370 C. The one drop of preparation was carefully placed into the test tube using a micropipette and 2 ml of gelation solution (composition 1 or 2) was added slowly[8].

Sterility test:

It was performed for aerobic, anaerobic and fungi microorganisms using fluid thioglycollate and soya bean casein digest medium as per IP 2007. Formulation took into laminar flow and passed through a membrane filter of 0.45µm with the help of vaccum pump. After filtration the filter paper was removed and cut into two halves. One half was dropped in fluid thioglycollate and other in soya bean casein digest. Both the media kept for incubation at 37°C for 7 days, and observed for any microbial growth[9].

Drug content uniformity:

The vials (n = 3) containing the preparation were shaken for 2–3 min manually and 100 µl of the preparation was transferred aseptically to 1ml of ethanol containing 25ml volumetric flasks with a micropipette and the final volume was made up with simulated tear fluid solution pH 7.4 at 35± 10c and sonicated for homogeneity. FSK concentration was determined at 210 nm by UV spectroscopy (Shimadzu, UV-1601, Japan) [10]

Intra ocular pressure (IOP) reduction studies:

The Forskolin (FSK) In situ gel formulations were tested for their IOP lowering effects on adult normotensive male New Zealand albino rabbits and the obtained results were compared with that of plain In situ formulations as well as plain FSK solution. An Increase in IOP was induced by the rapid infusion of a 5% glucose solution through the marginal ear vein. The amounts of injected were 15 ml/kg of body weight and infusion was accomplished in all animals within 30 sec. The IOP was measured by standardized Schiotz tonometer. Before the measurement of the tension, the cornea was anaesthetized with 2 or 3 drops of xylocaine (1% w/v). After 2 min, the eyelids were retracted gently with one hand, without exerting pressure on the eye ball. The lower cul-de-sac of right eye of each rabbit of the group (n = 4) received 25 µl of the optimized formulation while the contra lateral eye (left) received no drug and served as a control. The IOP of both eyes of each rabbit was measured immediately before the administration of formulation (zero reading), 30 min after instillation and then at every hour for a period of 8 h. The similar procedure was adapted for the measurement of IOP after instillation of 25 µl of plain FSK solution and plain In situ formulations, respectively. The change in IOP (ΔIOP) was determined by following equation:

$$\Delta IOP = IOP_{Dosed\ eye} - IOP_{Control\ eye}$$

RESULTS AND DISCUSSION

Extracted and isolated FSK was prepared in to solid dispersion (SD) with beta cyclodextrin (1:5) the SD was analyzed for solubility in tear fluid and observed that the solubility was increased. The full 23 factorial experimental design was to conduct a study of the effect of polymers like Carbopol 940(B), Sodium Alginate(C), Polaxomer F68 (a) and their interactions using a suitable statistical tool (Design-Expert® 9.0.3 Software) by applying ANOVA at 0.05 levels. The effects of the independent variables (a, B and C) on the gelation time (sec) and In vitro % drug release were evaluated, and the following models were obtained:

Table 2: Evaluation of Ocular In situ gel formulations

Formulation	Clarity	pH	Drug Content (%)	Gelling capacity
P1	clear	5.4±0.3	99.9±2	+
P2	clear	5.3±0.4	97.9±2	++
P3	clear	5.1±0.9	98.9±2	++
P4	clear	5.5±0.5	99.9±1	+++
P5	clear	5.8±0.2	98.9±2	+
P6	clear	5.6±0.6	97.9±2	+
P7	clear	5.9±0.7	99.9±2	++
P8	clear	5.7±0.6	97.9±2	+++

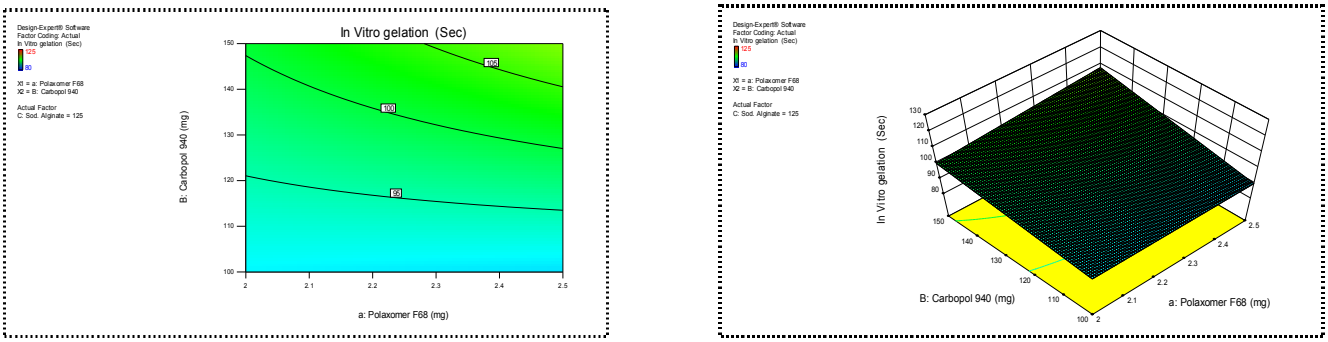

Figure 1: Effect of factors on in vitro gelation response; surface and contour plots.

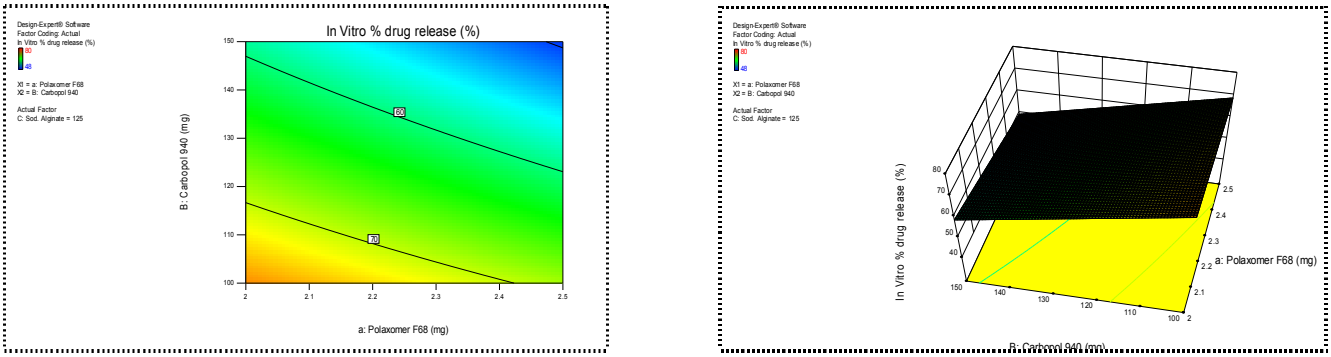

Figure 2: Effect of factors on in vitro % drug release; response surface and contour plots.

Figure 3: Sterility test for P8

From the ANOVA results of the model relating gelation time (sec) and In vitro % drug release as response, it can be noticed that all the coefficients of this model equations had statistical significance ($p < 0.05$). The three-dimensional response surface graph is very useful in learning about the main and interaction effects of the independent variables (factors), whereas two-dimensional contour plot gives a visual representation of values of the response.

All formulations are physically clear; it indicating there is no solid particles. pH of all formulations were ranging from 5.1±0.9 to 5.9±0.7. All formulations showed uniform drug content. P4 and P8 formulations showing good gelling capacity and retained for longer time.

The sterility test of optimized formulation was showed in fig.3. and the test was supporting the that all formulations were sterilized and there is no microbial growth upon storage.

The Intra ocular pressure (IOP) reduction studies suggested that the hypotensive activity of drug loaded In situ gel formulation (P8) was comparable to that of the plain drug solution. In the beginning, IOP decreased sharply for the first 2 h in case of plain FSK solution whereas IOP was observed to decrease slowly in case of drug loaded In situ gel formulation (P8). The IOP was immediately and noticeably reduced up to 2 h after instillation of plain FSK solution, but increased slightly over the rest of the period of observation. This type of fluctuation was not observed in case of optimized formulation, where the IOP continued to drop. The results suggested that In situ gel formulation (P8) produced a significant and prolonged reduction in IOP throughout 8 h.

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REFERENCES

1. Dubey, M.P., Srimal, R.C., Nityanand, S., and Dhawan, B.N., 1981. Journal of Ethnopharmacology. 3, 1–13.
2. Seamon, K.B., and Daly, J.W., 1981. Journal Cyclic Nucl Res. 7, 201–224.
3. Marone, G., Columbo, M., Triggiani, S., Vigorita, S., and Formisano, S., 1986. book of Agents Actions. 18, 96–99.
4. Meyer, B.H., Stulting, A.A., Muller, F.O., Luus, H.G., and Badian, M., 1987. Journal of South African medicine. 71, 570– 571.
5. Ammon, H.P.T., and Muller, A.B., 1985. Plant Medicine. 51, 473– 477.
6. Cohen, S., Lobel, E., Trevgoda, A., and Peled, Y., 1997. A novel in situ forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J Contr Rel. 44, 201-8.
7. Srividya, B., Cardoza, R.M., and Amin, P.D., 2001. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Contr Rel. 73, 205-211.
8. Nanjawade, B.K., Manvi, F.V., and Manjappa, A.S., 2007. In situ-forming hydrogels for sustained ophthalmic drug delivery. J Control Release. 122, 119-134.
9. Singh, V., Bushetti, S.S., Appala Raju, S., Rizwan, A., and Mamata, S., 2010. In vitro and in vivo evaluation of stimuli sensitive hydrogel for ophthalmic drug delivery. Ind J Pharm Edu and Res. 44(4), 380- 385.
10. Pandey, A., Prashant, Y.M., Sachdeva, D., Patel, D.K., and Ramesh, R., 2010. Development and optimization of levobunolol hydrochloride in-situ gel for glaucoma treatment. Int J Pharm and Bio Archives. 1(2), 134-139.

