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In vitro and *In vivo* Evaluation of Gellan Based Ocular Inserts of Phenylephrine

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Abstract

Gellan gum based ocular inserts of phenylephrine were prepared by solvent casting method, and evaluated for uniformity of thickness, weight, drug content, surface pH, *in vitro* release and *in vivo* mydriatic response in rabbits. The inserts were found to release drug following Higuchi square root release kinetics. *In vivo* comparison of the inserts with three times dosing of the conventional eye drop formulation revealed a comparable intensity and extent of mydriatic response produced by inserts. It can be concluded from the study that gellan gum based ocular inserts of phenylepherine can be effectively used for sustained topical ocular delivery.

Keywords: Gellan gum, phenylepherine, mydriatic response, ocular insert

Introduction

Gellan gum is an anionic heteropolysacchride produced aerobically by *Pseudomonas elodea* (Kang *et al.*, 1983). It consists of a linear tetrasaccharide repeat unit comprising of 1,3-β-D glucose, 1,4-β-D glucuronic acid, 1,4-β-D glucose and 1,4-L-rhamnose. Gellan gum forms gels on interaction with cations. Gellan gum has excellent ocular tolerance. Its film forming and gelling properties have been utilized for making scleral implants (Balasubramaniam *et al.,* 2004) and *in situ* gelling systems (Rozier *et al.*, 1989; Balasubramaniam *et al.*, 2003) for ocular delivery.

Phenylephrine, a pupil-dilating agent is commonly instilled into eye to produce mydriasis before an ophthalmic examination and surgery (Ho *et al.*, 1992). Topical delivery of drugs to eye is limited by rapid clearance of drugs from the cul-de-sac by tear turn over and blinking action. As a result to produce satisfactory mydriasis, frequent instillation is required, which is highly inconvenient to patients.

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Prolongation of precorneal residence by use of viscous solution (Seattone *et al.*, 1984), ointment (Hendrickson and Hanna, 1977), gel (Durrani *et al.*, 1996) and insert (Lee *et al.*, 1999) has been investigated for promoting the ocular availability of phenylephrine.

In the present investigation, gellan gum based ocular inserts of phenylephrine were prepared and evaluated for uniformity of thickness, weight, drug content , surface pH, *in vitro* release, and *in vivo* mydriatic activity.

Materials and Methods

Materials

Phenylephrine hydrochloride was gifted by Coax Bioremedies Pvt. Ltd, Hisar (India). Gellan gum was procured from Burzin and Leones Pvt. Ltd., Mumbai (India). HPLC grade methanol, water, and octane sulfonic acid were purchased from Qualigens Fine Chemicals, Mumbai (India). All other chemicals used were of reagent grade.

Preparation of ocular inserts

Ocular inserts of phenylephrine (1.5 mg/insert) were prepared by solvent casting method using mercury as the substrate (Mundada and Shrikhande, 2006). Required amount of gellan gum was dissolved in purified water at 80^0C, with the aid of stirring. To this glycerol (10 %, w/w) as plasticizer and calculated amount of phenylephrine were added, and stirred till homogenous. The solution was poured over a glass ring on the mercury surface and covered with an inverted funnel to allow slow and uniform evaporation at room temperature for 48 hr. The films so obtained were punched with the help of a sharp edged die.

Analysis of Phenylephrine

Reverse phase HPLC method was used for analysis of Phenylephrine. A 20 µl sample solution spiked with tropicamide (100 µg/ml) as an internal standard was injected into the chromatographic system (Waters, USA), equipped with 2487 dual λ UV detector, 600 pump controller and 7725*i* rheodyne injector. The chromatographic separation of phenylephrine was achieved in an isocratic run using methanol: water (0.1 % v/v octane sulfonic acid, 0.1 % v/v triethylamine, pH 3.0) [60:40] as mobile phase through Kromasil C8 5µm (125 x 4.6 mm i.d.) column. The flow rate was kept at 1ml/min, and eluant was monitored for the contents of phenylephrine at wavelength of 273 nm.

Evaluation of ocular inserts

Thickness, weight and surface pH: Thickness of the inserts was measured at 3 different spots using dead weight thickness gauze (Prolific, India). For uniformity of weight, 3 inserts from each batch were selected randomly and weighed individually on electronic balance (AND, Japan). Surface pH of the films was determined by pH indicator paper after moistening the inserts with 2 drops of purified water.

Content uniformity: Uniformity of the drug contents was determined by assaying the individual inserts. Each insert was grounded in a glass pestle mortar and 5 ml of methanol was added to make a suspension. The suspension so obtained was filtered through 0.45 µm syringe filter and 3 ml of the filtrate was taken in a sample vial and evaporated in air. The residue so obtained was dissolved in 100 µl of methanol and spiked with an aliquot of 20 µl of internal standard solution (tropicamide, 100µg/ml). The sample so obtained was analyzed for content of phenylephrine.

In vitro release studies: The inserts were placed in a beaker containing 100 ml of Sorenson phosphate buffer (pH 7.4) kept at 37 ± 1^0C and under constant stirring of 50 rpm (Sasaki et al. 1993). The inserts were covered with the wire net to prevent movement. At various intervals samples were withdrawn and analyzed for the content of phenylephrine.

In vivo mydriatic activity: The protocol for *in vivo* mydriatic study in rabbit was designed and an approval of institutional animal ethics committee (IAEC) was obtained. Three albino rabbits with equivalent pupil light response were used in the *in vivo* mydriatic study. Each rabbit was acclimated in the light of the laboratory for 1hr prior to the instillation of drugs. The animals were positioned in restraining boxes for instillation of drugs and after instillation the normal head and eye movements were allowed. The fabricated insert was placed carefully and gently in the lower cul-de sac of the right eye of the rabbit, while one drop of the marketed eye drop preparation was instilled into the left eye of the rabbit. The second and third dose of marketed eye drop preparation was further instilled at 15 and 30 min of the administration of first dose. Pupil diameter measurements were taken photographically at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min post instillation (Herrero-Vanrell *et al.*, 2000). The inserts were removed at the end of the experiment i.e. 6 hrs post instillation. Pupil diameters were measured from the pictures with a digital vernier caliper (Aerospace, China) with an accuracy of 0.02 mm.

The mydriatic response intensity, **I** was determined experimentally from the pupillary diameters, obtained at each time as per the following formula:

$$\mathbf{I = d_t - d_0}$$

Where $\mathbf{d_t}$ = the pupil diameter at time t following administration of the drug,

$\mathbf{d_0}$ = the pupil diameter at time zero.

The maximum change in pupil diameter (I_{max}), time to reach the I_{max} (t_{max}), and area under the mydriasis response vs. time curve (AUC) (calculated by trapezoidal rule), from 0 to 6 hr were evaluated.

Results and Discussion

The results of thickness, weight variation, surface pH and drug content uniformity are shown in Table 1. The inserts were found to possess uniform thickness and weight within the batch. It was found that the thickness and weight of inserts were increased with the increasing concentration of gellan gum, with batch A (0.25 % w/v, gellan gum) having thickness of 2.80 ± 0.004 µm and weight of 1.70 ± 0.02 mg, and batch E (1.5 % w/v, gellan gum)having thickness of 97.4 ± 0.002 µm and weight 13.39 ± 0.22 mg. The drug content was consistent in all batches and varied from 99.8 ± 0.10% to 96.9± 0.21%. As the surface pH of all the inserts was found to be approx. 7.5, they were not expected to cause irritation.

Table 1. Physiochemical characteristics of various batches of ocular inserts

Batch Code	Gellan Conc.(%,w/v)	Weight (mg)*	Thickness (µm)*	Surface pH	Content (%)*
A	0.25	1.70 ± 0.02	2.8 ± 0.004	7.5	99.8 ± 0.10
B	0.50	3.24 ± 0.37	6.3 ± 0.004	7.5	98.4 ± 0.30
C	0.75	5.65 ± 0.41	17.5 ± 0.003	7.5	98.2 ± 0.35
D	1.00	8.38 ± 0.16	38.6 ± 0.003	7.5	97.5 ± 0.59
E	1.50	13.39 ± 0.22	97.4 ± 0.002	7.5	96.9 ± 0.21

*Values are Mean ± SD (n = 3)

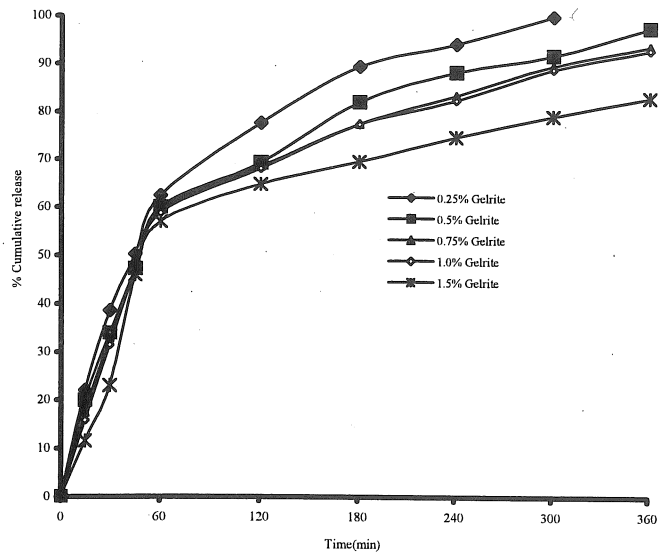

Figure 1. Comparative release profile of various batches of phenylephrine ocular inserts

Figure 1 compares the release profile of different batches of phenylephrine ocular inserts. It is evident from the results that there was a rapid initial release of phenylephrine from all the batches of inserts up to 60 min after which the release of drug slowed down. Ocular inserts of batch A (0.25 % w/v, gellan gum) released 99.82 % of drug at 5 hr interval and could not sustain the release up to 6 hr. As the concentration of gellan gum was increased the release rate slowed down and the remaining batches of ocular inserts could sustain the release up to 6 hr., and 95.99 %, 93.61 %, 92.88 % and 82.95 % of drug was released by ocular inserts of batch B, C, D and E respectively at the end of 6 hr. To determine the similarity between the release profile of different batches of ocular inserts, *in vitro* release data was compared using f_2 (factor of similarity) and f_1 (factor of dissimilarity) (Costa *et al.*, 2001). The release profiles are considered to be similar if $f_2 > 50$ and $f_1 < 15$, while values of $f_2 < 50$ and $f_1 > 15$ indicate dissimilarity between the profiles. The results of f_2 (63.32) and f_1 (7.73) show similarity between release profiles of batch A and batch B. On comparing release profile of batch B with C, D & E, the similarity was observed between B and C ($f_2 = 75.98, f_1 = 3.51$) and B and D ($f_2 = 71.76, f_1 = 4.67$), while the profiles of batch B and E were found to be dissimilar ($f_2 = 49.17$ and $f_1 = 15.09$).

To determine the release kinetics and mechanism of drug release, the release data was fitted into various kinetics models. The results of model fittings are shown in Table 2, it can be inferred from the data that the release of phenylephrine from ocular inserts follow Higuchi square root release kinetics, as the value of R^2 is maximum for Higuchi model. Further, the value of n ($n \approx 5$), the release exponent

of Korsmeyer-Peppas (Korsemeyer *et al.*, 1983) shows that the mechanism of release of phenylephrine from ocular insert is diffusion.

Table 2. Kinetic modeling of release data

Batch Code	R^2				
	Zero order	First order	Higuchi	Korsemeyer -Peppas	n
A	0.8571	0.7083	0.9476	0.9490	0.4466
B	0.8398	0.6813	0.9514	0.9333	0.4600
C	0.7930	0.6476	0.9255	0.9099	0.4741
D	0.8205	0.6294	0.9424	0.8981	0.4943
E	0.7413	0.5504	0.9007	0.9197	0.3437

n-release exponent of korsemeyer-peppas

Table 3. Mydriatic response characteristics of phenylephrine eye drop and ocular insert

S.No	Time (min)	Right Eye	Left Eye		
		Pupillary Diameter(mm)*	Pupillary Increment(I)*	Pupillary Diameter(mm)*	Pupillary Increment(I)*
1.	0	2.73 ± 0.08	0	2.33 ± 0.011	0
2.	15	3.23 ± 0.046	0.5 ± 0.036	2.63 ± 0.065	0.30 ± 0.049
3.	30	3.70 ± 0.064	0.97 ± 0.050	3.23 ± 0.070	0.90 ± 0.012
4.	45	4.50 ± 0.049	1.77 ± 0.040	3.89 ± 0.046	1.57 ± 0.087
5.	60	4.59 ± 0.061	1.87 ± 0.023	3.95 ± 0.071	1.62 ± 0.085
6.	90	4.68 ± 0.032	1.95 ± 0.051	4.03 ± 0.085	1.7 ± 0.108
7.	120	4.76 ± 0.040	2.03 ± 0.069	4.22 ± 0.040	1.89 ± 0.096
8.	180	4.58 ± 0.089	1.85 ± 0.123	3.98 ± 0.046	1.65 ± 0.104
9.	240	4.35 ± 0.065	1.62 ± 0.015	3.75 ± 0.091	1.42 ± 0.167
10.	300	3.86 ± 0.015	1.13 ± 0.086	3.56 ± 0.026	1.23 ± 0.131
11.	360	3.77 ± 0.015	1.05 ± 0.065	3.24 ± 0.053	0.91 ± 0.132

* Values are Mean ± SD (n = 3)

The phenylephrine ocular inserts of batch B which provided the optimum *in vitro* release of phenylephrine for 6 hr were selected for the *in vivo* study. This time period of 6 hr is sufficient for a routine eye examination as well as for cataract surgery. The inserts used *in vivo* were casted aseptically from sterile solution. Table 3 shows the mean pupillary diameter and mean increment in pupillary diameter of the eyes treated with phenylephrine 10 %, w/v eye drops (Drosyn®, FDC Ltd., Mumbai) and ocular inserts. Figure 2 represents the increase in pupillary size of phenylephrine treated eyes as a function of time. The results show that it took 45 min to reach the steady state pupil diameter and 120 min (t_{max}) to reach the maximum pupillary response i.e. maximum pupillary increment (I_{max}). The mean I_{max} was 2.03 ± 0.069 mm in insert treated eyes and 1.89 ± 0.096 mm in eye drop treated eyes which translate into an average increase in cross sectional area of pupil i.e. viewing area of 3.23 mm^2 and 3.0 mm^2 respectively. The area under the mydriatic response vs. time curve (AUC) was calculated to be 1530.35 mm x min and 1338.47 mm x min for insert and eye drop treated eyes respectively. Thus there was a slight increase in I_{max} and AUC in insert treated eyes compared to eye drop treated eyes. On applying student's t-test no significant ($p > 0.05$) difference was observed in I_{max} and AUC of insert and eye drop treated eyes.

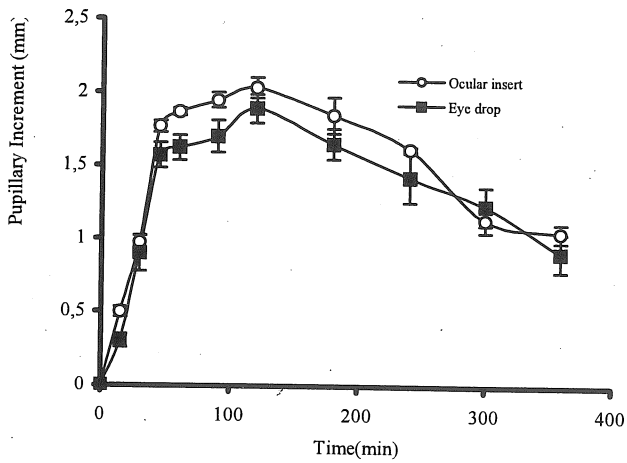

Figure 2. Mean mydriatic response as a function of time following instillation of three drops of phenylephrine eye drop and ocular insert

Conclusion

The present investigation was undertaken with the objective of preparing a sustained release ocular delivery system of phenylepherine using gellan gum as the biopolymer. *In vitro* release studies revealed that the sustained release of phenylepherine followed the Higuchi square root release pattern. *In vivo* comparison of the optimized batch of inserts with the three time dosage regimen of the conventional eye drop formulation revealed a comparable mydriatic response of

the ocular insert. The study indicates potential usefulness of the gellan gum based ocular insert for controlled ocular delivery of phenylephrine.

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