Evaluation of Dipivefrin Hydrochloride Entrapped Non-Ionic Surfactant Based Gels for The Treatment of Ocular Disease

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ABSTRACT

The present study was aimed on developing and characterizing niosomal gels loaded with adrenergic agonist; dipivefrin HCl for prolonging precorneal residence time and improving bioavailability of drug for glaucoma treatment. Dipivefrin HCl niosomes were prepared using various non-ionic surfactants (span 20, span 60 and span 80) in the presence of cholesterol in different molar ratios by ether injection method. The selected formulations were incorporated into carbopol 934 and locust bean gum-based gels. TEM studies confirmed that niosomes formed were white and spherical in shape and have a definite internal aqueous space with uniform particle size. Formulation F4 composed of span 60 and cholesterol (1:1) gave the highest entrapment (92.16±0.25%) and slower release results after 8 hours (Q8h=61.05±2.87%) among other formulations. The in-vitro drug permeation studies showed that there was a slow and prolonged release of drug from niosomal gel formulations as compared to niosomes itself. Considering the in-vitro release, niosomal gel formulation G2 were the best among the studied formulations. No sign of redness, inflammation, swelling or increased tear production was observed by Draize test. The IOP lowering activity of selected formulation was detected and compared with marketed Pilopine HS® gel. G2 formulation showed relative bioavailability 2.64 times more than bioavailability of marketed Pilopine HS® gel.

Keywords: Niosomes, Dipivefrin HCl, Niosomal gel, Draize test, IOP, antiglaucomatic activity.
DOI Number: 10.48047/NQ.2022.20.13.NQ88524 **Neuroquantology 2022; 20(13):4305-4313**

INTRODUCTION

Glaucoma is a prevalent neurodegenerative disorder of the eye. Increased intraocular pressure (IOP) and subsequent retinal ganglion cell (RGC) death leading to the loss of visual field characterizes the pathology of primary open angle glaucoma (POAG), which is the most common form. The disease affects over 66 million people worldwide, causing bilateral blindness in 6.8 million (1, 2). Patients with POAG typically exhibit increased resistance to the outflow of aqueous humor through the trabecular meshwork, which can result in an increase in IOP and subsequent cell death from compression of the optic nerve axons (3). However, IOP is the primary risk factor causing the loss of RGCs; the strategies of treatment mostly involve lowering IOP (4). Current treatment options primarily aim at decreasing IOP by utilizing pharmacological agents, laser

therapy and surgery. The method of reducing IOP is by enhancing the outflow of humor from the eyes using muscarinic acetylcholine receptor agonists (5, 6).

Dipivefrin (DV) HCl, a prodrug of epinephrine (EP), is an adrenergic agonist and direct acting sympathomimetic agent that are used to reduce IOP in patients suffering from chronic open angle glaucoma (7). This drug acts through decreasing production and increasing the outflow of aqueous humor from the eye (8). A controlled study proved the usefulness of topically applied DV (0.1%, w/v) over EP (2%, w/v) in reducing the IOP in the patients who were intolerant to topically applied EP (9). In terms of safety, DV is associated with less systemic adverse effects (e.g., cardiovascular side effects) compared to EP, since it is only needed in very small dose. Thus, DV is considered more suitable for ocular application as compared to EP, especially in patients with cardiovascular disorders (10). In addition to the clinical benefits, DV has favorable physicochemical properties compared to EP. DV has an ideal lipophilicity and diffusivity across the lipophilic ocular dynamic and static barriers, due to the esterification of the two hydroxide (-OH) functional groups of EP, yielding dipivaloyl-EP. This chemical modification allows DV to avoid the unfavorable physicochemical and biopharmaceutical characteristics of the EP (11). Therefore, using DV in an ocular formulation will resolve the lipophilicity issue associated with EP and would provide a site-specific delivery with a 10-fold enhanced therapeutic efficacy compared to EP (12). Delivering drugs via the ocular route is challenging due to the immediate tear-turnover rate and corneal impermeability, which restricts the ocular bioavailability of conventional topical eye drops or solutions (13). Therefore, there is a need for an appropriate ocular delivery system to achieve high transcorneal permeation, sustained and controlled delivery while providing sufficient ocular bioavailability (14). These problems can be minimized using niosomal vesicular system.

Niosomes are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures, which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane (15). Niosomes, administered as an ophthalmic gel, are capable of localizing and maintaining drug activity at its site of action due to their easy transition through ocular barrier with reduced drug frequency and toxicity. Moreover, niosomes based ocular gel containing bioadhesive polymer helps the drug to remain adhered to corneal surface for a long period of time. Hence, precorneal residence time is increased, resulting in significant enhancement of ocular bioavailability.

Therefore, the present study aims to develop and evaluate niosomal gel formulations containing dipivefrin HCl to achieve prolonged precorneal residence time and improved bioavailability. Niosomes were prepared using various non-ionic surfactants (span 20, span 60 and span 80) in the presence of cholesterol in different molar ratios by ether injection method. Selected formulations of niosomes were incorporated into carbopol 934 (1% w/w) and locust bean gum (3% w/w) gels.

MATERIALS AND METHODS

The dipivefrin HCl was kindly received as a gift sample by M/s Piramal Enterprises Ltd. (Digwal AP, India). Sorbitan monolaurate (span 20), sorbitan monosterate (span 60), sorbitan monooleate (span 80), cholesterol, locust bean gum and carbopol 934 were procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Isopropanol, methanol, acetone, chloroform, boric acid, sodium hydroxide, sodium bicarbonate, potassium chloride, glacial acetic acid, magnesium, sodium chloride, calcium chloride dehydrate, potassium dihydrogen

eISSN1303-5150 www.neuroquantology.com

phosphate and disodium hydrogen phosphate were purchased from were purchased from S.D Fine chemicals (Mumbai, India). Double distilled water was used throughout the study.

PREPARATION OF DIPIVEFRIN HYDROCHLORIDE LOADED NIOSOMES

Dipivefrin hydrochloride loaded niosomes were prepared by ether injection technique using non-ionic surfactants (span 20, span 60 and span 80) and cholesterol in different ratios as shown in Table 1. For each ratio, non-ionic surfactant and cholesterol were weighed accurately and dissolved in 20 ml of diethyl ether. Dipivefrin hydrochloride (10 mg) was then dissolved in this lipid solution. The resulting solution was taken in a syringe and injected slowly through a 16 gauge needle into 10 ml of aqueous phase (phosphate buffer saline pH 7.4) held in a beaker maintained at 60^0C to 65^0C and agitated slowly. As the lipid solution was injected slowly into the aqueous phase, vaporization of diethyl ether resulted in the formation of niosomes. The prepared niosomes were separated by ultracentrifugation (Remi C-24, Mumbai, India) at 4^0C.

PREPARATION OF GELS CONTAINING DIPIVEFRIN HCl NIOSOMES

Selected drug loaded niosomes (equivalent to 0.1% w/w drug) were incorporated into different gel bases. The polymers used were locust bean gum and carbopol 934. The prepared niosomal gels were investigated for isotonicity, *in-vitro* drug permeation and stability study as discussed earlier by Jain *et al.,* 2020 method (16). Composition for niosomal gels were shown in Table 2.

EVALUATION OF NIOSOMAL GELS

***Ex-vivo* drug permeation study**

Freshly excised whole cow eyeball was procured from a slaughter house and transported to the laboratory in cold condition. They were maintained in normal saline at 4°C. The cornea was then carefully removed along with a 5-6 mm of surrounding scleral tissue and washed with cold saline. The washed cornea was preserved in freshly prepared phosphate buffer (pH 7.4) and stored under refrigeration until the time of the study. The preserved cornea was mounted on Franz diffusion cell by sandwiching between the donor and receptor compartment. It was positioned on the donor half-cell such that the epithelial surface was facing the donor solution. The receptor half-cell was positioned symmetrically opposing the donor half-cell. The half-cells were secured together with a clamp. This procedure prevents any leaks (17).

One gram of selected niosomal gel formulation (G2) was placed inside the donor half-cell over the corneal membrane. The entire surface of the cornea was in contact with the receptor compartment that contained 50 ml of simulated tear fluid (pH 7.4), which was stirred continuously using a magnetic stirrer at 100 rpm to simulate blinking action. At predetermined time intervals for up to 8 hr, 5 ml aliquots of the release medium were withdrawn for analysis and were replaced with equal volume of release medium at the same temperature to maintain constant volume. *Ex-vivo* drug permeation through cornea from niosomal gel was analyzed spectrophotometrically using UV-spectrophotometer at 254 nm and compared with marketed formulation (Pilopine HS® gel). Results were tabulated and graph was plotted as cumulative percentage of drug permeated versus time for niosomal gel formulation (G2). Study was done in triplicate.

Ocular irritancy test (Draize's test)

Rabbits were divided into two groups (four rabbits in each group). G2 niosomal gel formulation and marketed Pilopine HS® gel

were applied to Group I and Group II of rabbit's eyes respectively. The untreated eye serves as control. 20 µl of the representative formulation was instilled into the lower conjunctival sac of the rabbit's right eye, while the left was kept as a control. The solutions were instilled periodically twice a day. The test eyes were examined for any abnormality (irritation signs) that were recorded before treatment and 30 min, 1 h, 24 h, 48 h, 72 h, 7 days, 14 days and 21 days after treatment (18). The common irritation signs are expected to be conjunctival redness, swelling and discharge scoring 0 (absence) to 4 (highest).

***In-vivo* antiglaucoma activity by measurement of intraocular pressure (IOP)**

Rabbits were randomly divided into three groups (six rabbits in each group). Group I served as control while Group II and Group III were treated with G2 niosomal gel formulation and marketed Pilopine HS® gel respectively (19, 20). Glaucoma disease was induced by Bonomi *et al.*, 1978 method (21). Rabbits were treated with subconjunctival injections of 0.25 ml Betamethasone injection (Betamethasone sodium 4 mg/ml) every week for three successive weeks in left and right eyes. Local anesthetic eye drops (Benox®) were used prior to subconjunctival injection. The activity was confirmed by noticing a bulge formation at the site of injection. The right eye of each rabbit was kept as control and the left eye was treated for glaucoma using 10 µl (equivalent to 10 µg of Dipivefrin HCl) of selected formulation (G2) and marketed Pilopine HS® gel for group II and group III respectively. The intraocular pressure (IOP) readings were measured using Schiotz Tonometer, before drug administration and 2 h, 4 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h and 24 h after drug administration. IOP was measured three times at each time interval and the means were recorded.

The change in IOP (ΔIOP) is expressed as follows:

ΔIOP = IOP dosed eye – IOP control

The pharmacokinetic parameters taken into consideration such as the maximum percentage decrease in IOP (% IOP_{max}) and the time of maximum response (T_{max}) were estimated through constructing % ΔIOP versus time curves.

$$\%\ \text{Maximum reduction of IOP}\ (\%\ \text{IOPmax}) = \frac{\text{IOPo} - \text{IOPmax}}{\text{IOPo}}\ \text{X}\,100$$

Where, IOP_o is the intra-ocular pressure at 0 time, IOP_{max} is the intra-ocular pressure at $T_{max.}$ The area under the curve (AUC) was calculated according to trapezoidal rule. Relative bioavailability for formulations was determined through the equation below:

$$\text{Relative bioavailability} = \frac{\text{AUCt}}{\text{AUCs}}$$

Where, AUCt and AUCs are the AUC estimated for test formulation and standard formulation (marketed Pilopine HS® gel).

RESULTS AND DISCUSSION

***Ex-vivo* drug permeation study**

The *ex-vivo* permeation of dipivefrin HCl from selected formulation G2 and marketed dipivefrin formulation (Pilopine HS gel) through

bovine cornea was determined in triplicate and their mean values with standard deviation was shown in Table 3 and the plot of cumulative percent drug permeated as a function of time was shown in Figure 1. The results showed slow and sustained release of drug through the corneal membrane for prolonged period of time in case of niosomal gel formulation (G2) i.e. 74.21±1.05% of drug release in 8 h as compared to marketed formulation i.e. 81.47±0.24% of drug release in 8 h. Faster release of dipivefrin HCl from the marketed gel may be due to the free drug being present in gel structure as compared to niosomal gel in which the drug was entrapped into niosomal vesicular structure. These results were in agreement with Asthana *et al.*, 2016 (22). It has been stated that more hydrophobic span surfactants form more compact niosomes when hydrated in presence of cholesterol (23).

Ocular irritancy test (Draize's test)

The possibility of eye irritation due to selected niosomal gel formulation (G2) and marketed formulation instillation were evaluated in rabbits. No signs of redness, inflammation, swelling or increased tear production were observed over the study period for tested formulation as shown in Figure 2. No ophthalmic damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible. This indicated that the non-ionic surfactants namely span 60 as well as cholesterol used in the niosome formulations were non-irritant to the eye and could be used safely. By instillation of marketed Pilopine HS® gel, the rabbit's eye showed irritation, redness and inflammation at conjunctiva which may be due to its large molecular size, indicating a problem in the conjunctival absorption of drug as shown in Figure 3.

***In-vivo* antiglaucoma activity by measurement of intraocular pressure (IOP)**

As shown in Table 4, it was observed that marketed formulation showed a decrease in IOP up to 17.42±1.12 mmHg at the end of 7 h, but then there was an increase in the IOP, which may be due to the elimination of the drug from the site of action. Hence, it was unable to sustain the activity for a long period of time, which calls for frequent administration of the formulation. G2 decreased IOP by 16.99±1.25 mmHg at the end of 9 h. G2 maintain the sustained effect up to 24 h. This control of IOP for prolonged periods may be attributed to the increased corneal residence and sustained drug release of the formulated ophthalmic niosomal gel compared to marketed Pilopine gel. It was also observed that upon administration of dipivefrin HCl ocular niosomal gel, no effect on IOP in the control eye, this may be an indication that no systemic absorption occurred. It was revealed that the sustained effect was maintained for more time in the niosomal gel as compared to the marketed formulation. The marketed Pilopine gel lowered the IOP to minimum (16.32±1.11 mmHg) and afterwards, there was a sudden increase in the IOP upto 40.01±0.43 mmHg in 24 h whereas, niosomal gel lowered the IOP slowly to the minimum and thereafter, a gradual increase in the IOP upto 21.93±1.24 mmHg in 24 h was observed.

The mean percentage decrease in IOP after installation of single dose of either niosomal gel formulations (G2) or marketed formulation was calculated and the data was shown in Figure 4. The mean pharmacokinetic parameters for niosomal gel G2 and marketed Pilopine gel was listed in Table 5. The ΔIOP_{max} values for G2 and marketed formulation were 22.49±0.06% and 22.65±1.12% respectively. T_{max} values were 9 h and 7 h respectively. Greater AUC values were observed for G2 (596.99 mmHg.h) compared with marketed Pilopine gel (225.34 mmHg.h). The relative bioavailability for G2 to marketed formulation was 2.64.

Higher bioavailability in case of G2 confirmed that the niosomal gel formulation had a prolonged duration of its anti-glaucomatic effect in comparison to the marketed Pilopine gel.

CONCLUSION

The results of this study showed that there was a slow and prolonged release of drug from selected niosomal gel formulation G2 as compared to marketed formulation (Pilopine HS® gel). No sign of redness, inflammation, swelling or increased tear production over the study period for selected gel formulation was observed by Draize test. Niosomal gel formulation G2 gave higher AUC than that given by marketed Pilopine HS® gel and increased the bioavailability by 2.64 times than marketed Pilopine HS® gel. These results suggest that the niosomal gels containing dipivefrin HCl are promising ocular carriers for glaucoma treatment.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This protocol was approved by the Institutional Animal Ethical committee of Department of Pharmacy, B.R. Nahata College of Pharmacy, Mandsaur (M.P.)

ACKNOWLEDGEMENTS

The authors are thankful to the Principal, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, UP, India, for their kind support and providing all the necessary facilities and encouragement for successful completion of this work.

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TABLES AND FIGURES

Table 1: Composition for niosomes

Formulation Code	Surfactant	Amount of surfactant (mg)	Cholesterol (mg)	Drug (mg)	Ratio (Surfactant: Cholesterol)
F1	Span 20	100	100	10	1:1
F2	Span 20	200	100	10	2:1
F3	Span 20	100	200	10	1:2
F4	Span 60	100	100	10	1:1
F5	Span 60	200	100	10	2:1
F6	Span 60	100	200	10	1:2
F7	Span 80	100	100	10	1:1
F8	Span 80	200	100	10	2:1
F9	Span 80	100	200	10	1:2

Table 2: Composition for niosomal gels

Gel formulation	Niosomes loaded	Locust bean gum (% w/w)	Carbopol 934 (% w/w)
G1	F1	3%	1%
G2	F4	3%	1%
G3	F7	3%	1%

Table 3: Results of percent cumulative drug permeated from niosomal gel formulation (G2) and marketed formulation in STF of pH 7.4

Time (hr)	Average percent cumulative drug permeated (Mean ± SD, n=3)	
	G2	Marketed formulation
0	0	0
1	06.97±1.23	10.95±0.34
2	12.65±0.86	14.15±0.76
3	20.81±1.98	23.97±0.43
4	32.46±1.17	38.64±1.45
5	42.12±0.24	47.71±1.21
6	52.75±1.65	57.94±0.97
7	65.51±0.67	69.29±0.56
8	74.21±1.05	81.47±0.24

Table 4: IOP lowering effects of G2 and marketed formulation after treatment

	IOP (mmHg) lowering effects of G2 and marketed formulation

Time (hr)	Group II (G2)		Group III (marketed Pilopine HS gel)	
	R	L	R	L
0	40.47±0.42	40.47±0.04	40.07±0.19	40.07±0.02
2	40.47±0.42	35.12±0.17	40.07±0.19	30.95±1.68
4	40.47±0.42	27.89±0.28	40.07±0.19	27.37±2.32
6	40.47±0.42	23.85±0.31	40.07±0.19	21.69±0.45
7	40.47±0.42	21.20±0.53	40.07±0.19	16.32±1.11
8	40.47±0.42	19.28±0.06	40.07±0.19	20.95±3.21
9	40.47±0.42	16.99±1.25	40.07±0.19	21.69±0.57
10	40.47±0.42	19.54±2.31	40.07±0.19	24.23±1.12
11	40.47±0.42	20.94±0.96	40.07±0.19	28.96±4.96
24	40.47±0.42	21.93±1.24	40.07±0.19	40.01±0.43

Table 5: Pharmacokinetic parameters for G2 and marketed Pilopine gel

Formulations	IOP_{max} (%)	T_{max} (hr)	$AUC_{0-24\ h}$ (mmHg.h)
G2	22.49±0.06	9	596.99
Marketed Pilopine gel	22.65±1.12	7	225.34

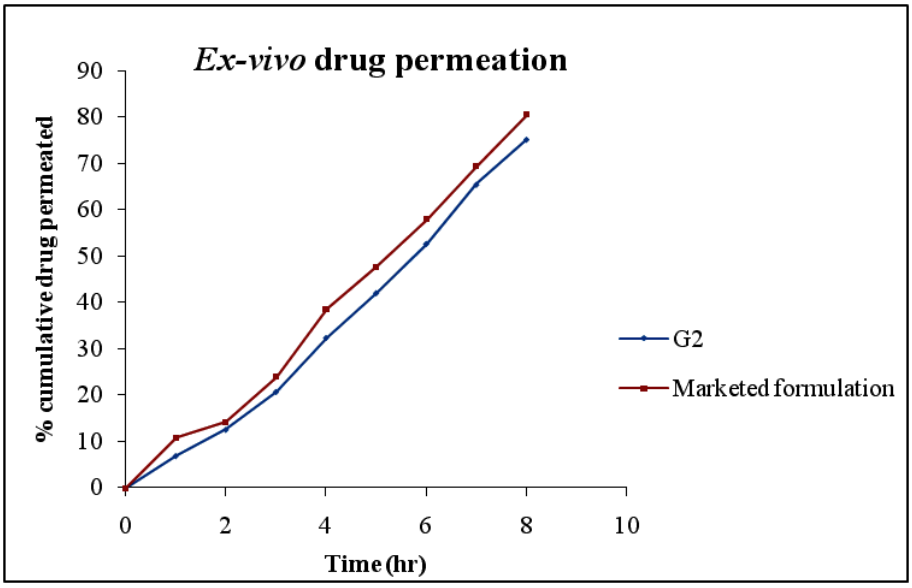

Figure 1: *Ex-vivo* permeation study of selected formulation (G2) and marketed formulation

Figure 2: Representative illustrations of ocular irritation test for selected formulation (G2)

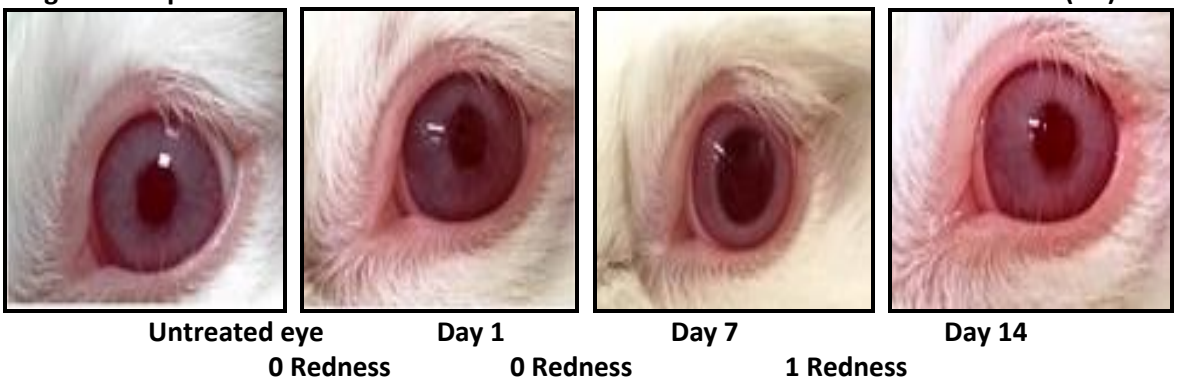

Figure 3: Representative illustrations of ocular irritation test for marketed formulation

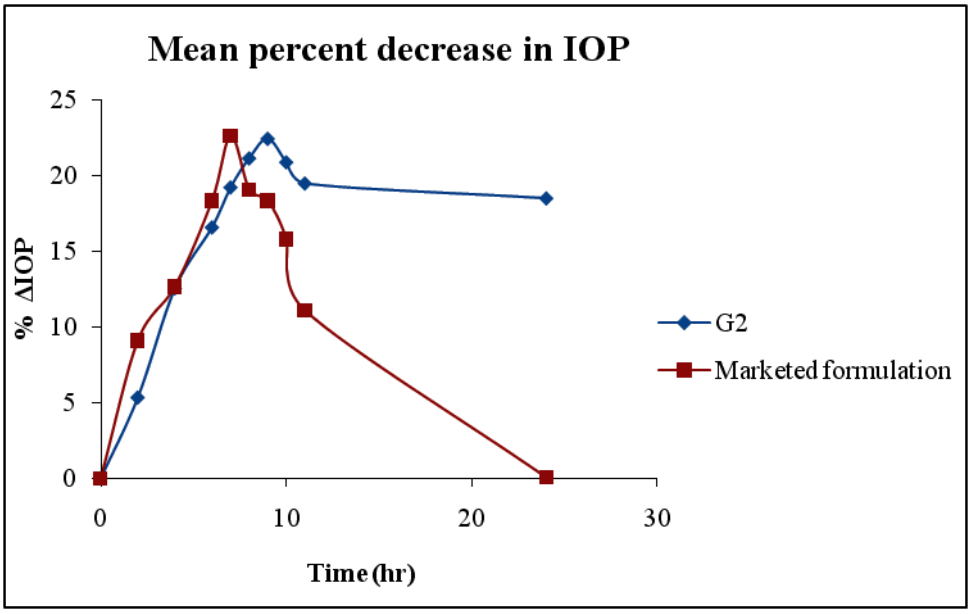

Figure 4: Mean percentage decrease in intraocular pressure (% ΔIOP) versus time