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Design and *In-vitro* Characterization of Levobunolol Hydrochloride Occuserts with Special Reference to Glaucoma Treatment

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ABSTRACT

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The aim of present study was to design and *in-vitro* characterization of Levobunolol HCl occuserts with special reference to glaucoma treatment. Levobunolol HCl ophthalmic solution has been shown to be effective in IOP and may be used in patients with chronic open-angle glaucoma or ocular hypertension. The Levobunolol HCl occuserts were prepared using hydroxypropyl methylcellulose (HPMC, 3 & 4%), poly vinyl pyrrolidone (PVP, 1%), methyl cellulose (MC, 1 & 2%) as polymers by solvent casting technique with objectives of increasing contact time, achieving controlled release, reduction in frequency of administration and greater therapeutic efficacy. The prepared occuserts were then evaluated for there physical appearance and surface texture, weight variation, uniformity of thickness, tensile strength and % elongation at break, % moisture uptake, % moisture loss, folding endurance, drug content and *in-vitro* drug release. It is evident from these studies that, formed polymeric occuserts have appreciable strength and safety. Physiochemical characterization and *in-vitro* drug release studies reveals that, the prepared occusert formulations (F_2 & F_1) containing 4 % and 3 % HPMC had released their drug content, 83 % and 81 % respectively over an extended period of 12 hr. Hence, these formulations were selected as best optimized formulations.

Keywords: Levobunolol HCl, Occusert, Glaucoma, *In-vitro* characterization

INTRODUCTION

Glaucoma is a group of diseases of eye characterized by damage to the ganglion cells and optic nerves. Glaucoma is usually described as open angle and/or closed angle (angle closure). These terms are based upon the mechanism of obstruction of outflow of aqueous humor and help clinicians for developing treatment strategies. A third type is congenital glaucoma, which results from developmental ocular abnormalities and occurs in less than 2% of patients[1]. Among asians, the incidence of closed angle glaucoma is almost twice as high as that for caucasians. The true picture of disease incidence in asian continent remains to be elucidated as yet. Current studies indicate the involvement of excitatory and inhibitory neurotransmitters *viz.* glutamate, gamma amino butyric acid (GABA) and glycine in the development of glaucoma. The excess supply of excitatory neurotransmitter glutamate is particularly linked to glaucoma. Apoptosis or genetically programmed cell death has also been implicated as a mechanism for progression of glaucoma[2]. Levobunolol HCl is a non-cardioselective β-adrenoceptor blocking agent, equipotent at both β_1 and β_2 receptors. Levobunolol HCl does not have significant local anesthetic (membrane-stabilizing) or intrinsic sympathomimetic activity. β-adrenergic receptor blockade reduces cardiac output in both healthy subjects and patients with heart disease. The primary mechanism of ocular hypotensive action of levobunolol HCl in reducing intra-ocular pressure (IOP) is most likely a decrease in aqueous humor production. Levobunolol HCl ophthalmic solution has been shown to be effective in IOP and may be used in patients with chronic open- angle glaucoma or ocular hypertension. Its side effects are transient burning sensation, blurred vision, mild ocular irritation, bronchospasm, reduction in resting heart rate and blood pressure[3]. Previous literature on Levobunolol HCl reported that, only *in-situ* gels were prepared by *pH* induced gelling system for glaucoma treatment[4]. But no attempt has been found to substantiate their property such as lowering intraocular pressure for glaucoma treatment. Therefore, our aim is to design and develop Levobunolol HCl polymeric occuserts by solvent casting technique with special reference to glaucoma and their multi-system complications.

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MATERIAL AND METHODS

Chemicals:

Levobunolol HCl was procured from BAL Pharma Ltd., Bangalore, Hydroxy Propyl Methyl Cellulose (HPMC 15cps) from Colorcon Ltd., Goa and Methyl Cellulose (MC), Ethyl Cellulose (EC), Poly Vinyl Pyrrolidone (PVP K32) and Dibutyl Phthalate (DBP) from S. D. Fine Chemicals, Bombay. Solvent used was distilled water, which is permissible for eye delivery. All other reagents and solvent used for study were of analytical grade.

Instruments:

U.V. Visible Spectrophotometer-1700 (Shimadzu, Japan), Digital Balance (Shimadzu, Japan), Magnetic Stirrer, Overhead Stirrer and Cyclone Mixer (Remi, Mumbai), Hot Air Oven (Tempo Pvt. Ltd., Mumbai) instruments were used for this study.

PREPARATION OF LEVOBUNOLOL HCl POLYMERIC OCCUSERTS:

Selection of best polymer composite for occuserts:

Polymers were selected on the basis of their solubility or dispersibility and compatibility with solvents. Solvent volatility was also taken into consideration.

Preparation of blank polymeric occuserts:

The various formulations of blank polymeric occuserts were prepared using various concentrations of HPMC, MC and PVP both alone and in combination as shown in **Table-1,** by solvent casting technique[5,6].

Incorporation of drug:

The best polymeric blank occuserts were used for incorporation of pure Levobunolol HCl drug. Calculated amount of Levobunolol HCl was dispersed in the polymeric solution along with glycerine as a plasticizer to form a uniform dispersion. The air bubble produced during dispersion was removed by subjecting the solution for sonication. The dispersion was casted on glass plate using a ring 4 cm diameter having (an area of 12.5714cm^2) 3 ml capacity[7,8]. The calculations were as follows.

Calculation:

Diameter of the proposed occusert (d) = 0.6 cm

Radius of the proposed occusert (r) = 0.3 cm

Therefore, Area of the proposed occusert (A) = πr^2 = 0.2828 cm^2

Diameter of the ring (d) = 4 cm

Radius of the ring (r) = 2 cm

Total area of the ring (A) = πr^2 = 12.5714 cm^2

Capacity of the ring = 3 ml

No. of occuserts present in the proposed area of the ring = Total area of ring / Area of one occusert = 12.5714 / 0.2828 = 44 occuserts.

Total amount of drug added to the polymer solution = 28.16 mg

One occusert contains = 28.16 / 44 = 0.64 mg

Amount of drug present in one occusert = 0.64 mg of Levobunolol HCl

After drying, circular films of occuserts were cut into diameter of 6 mm, each containing 0.64 mg of Levobunolol HCl and stored in an airtight container (dessicator) under

Table 1: Compositions of different optimized Levobunolol HCl occusert formulations.

FC	Drug	Drug Reservoir (%)				RCM (%)	
	Levobunolol	Film Former*			Plasticizer**	Film Former*	Plasticizer**
	HCl (Mg)	HPMC (%)	PVP (%)	MC (%)	Glycerine (%)	EC (%)	DBP (%)
F_1	0.64	3	-	-	40	6	30
F_2	0.64	4	-	-	40	6	30
F_3	0.64	3	1	-	40	6	30
F_4	0.64	4	1	-	40	6	30
F_5	0.64	-	-	1	40	6	30
F_6	0.64	-	-	2	40	6	30
F_7	0.64	-	1	1	40	6	30
F_8	0.64	-	1	2	40	6	30
F_9	0.64	1	-	1	40	6	30
F_{10}	0.64	1	-	2	40	6	30

*Based on total volume of solvents; **Based on total polymer weight; FC- Formulation code; RCM - Rate Controlling Membrane.

ambient condition during the course of study. Finally, it is packed in a well closed light blue high intensity containers with polystyrene caps and protected from light[3, 9].

Formulation of rate controlling membrane:

The rate controlling membrane was casted on a glass plate using ethyl cellulose (6%), which is dispersed in chloroform and dibutyl phthalate (30 % w/w of polymer) as plasticizer. Circular membranes of 8 mm diameter were cut using a special mould[10]. Both the drug reservoir and rate controlling membrane was sealed to control release of drug from periphery.

EVALUATION OF LEVOBUNOLOL HCl OCCUSERTS:

Physical appearance and surface texture:

It includes visual inspection as well as texture appearance by feel or touch.

Weight variation:

Weight variation test was done by taking five occuserts from each batch and their individual weights were determined by using a digital electronic balance[11].

Uniformity of thickness:

The thickness of occuserts was measured using a screw-gage micrometer with a least count of 0.01mm at five different spots of occuserts and average was taken[12].

Tensile strength and % elongation at break:

Occuserts having 6 cm in length and 1cm in width were cut and held between two pairs of acrylic slides with the help of clamps. One pair of acrylic slide grips upper end of occusert stripes, while other pair to another end by hanging a flat pan (for adding weight) with the help of a metal wire. The tensile strength and % elongation at break can be conveniently observed with the help of traveling microscope. The rate of change of stress was kept constant by increasing the load on flat pan at rate of 10 g/ 2 min., because stress strain relationship changes with rate of changes in stress[13]. The % elongation at break were calculated by using formula,

$$\% \text{ elongation at break} = I_B - I_o / I_o \times 100$$

Where, I_B = Length of occuserts at break, when stress is applied.

I_o = Original length of occuserts.

% moisture uptake:

Occuserts were weighed and kept in a dessicator containing 100 ml of saturated solution of aluminum chloride by which a relative humidity 79.5 % was maintained. After 3 days, occuserts were taken out and reweighed[12]. The % moisture uptake was calculated by using formula,

$$\% \text{ moisture uptake} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

% moisture loss:

Occuserts were weighed and kept in a dessicator containing anhydrous calcium chloride. After 3 days, the occuserts were taken out and reweighed[14]. The % moisture loss was calculated using the formula,

$$\% \text{ moisture loss} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Folding endurance:

Folding endurance of occuserts was determined by repeatedly folding a small strip of occuserts (approximately 2.0 x 2.0 cm) at the same place until it broke. The number of times occuserts could be folded at the same place without breaking gives the value of folding endurance[15].

Drug content studies:

Five occuserts were taken from each batch and dissolved or crushed in 10 ml of isotonic phosphate buffer (pH 7.4) in a beaker and were filtered into 25 ml volumetric flask and volume was made up to the mark with buffer. 1 ml of the above sample was withdrawn and absorbance was measured by U.V Visible Spectrophotometer at 258 nm after suitable dilutions[16].

***In-vitro* drug release:**

In-vitro drug release from different occuserts was studied by using classical standard cylindrical tube (internal diameter 15 mm and height 100 mm) fabricated in laboratory. The diffusion cell membrane was tied to one end of open cylinder, which acted as donor compartment. Occuserts was placed inside this compartment. The diffusion cell membrane acted as corneal epithelium. The entire surface of membrane was in contact with the receptor compartment containing 50 ml isotonic phosphate buffer (pH-7.4) in 100 ml beaker. The content of the receptor compartment was stirred continuously using magnetic stirrer and temperature was maintained at 37 ± 0.5 °C. At specific intervals of time, 1 ml of sample was withdrawn from the receptor compartment and replaced with equal volume of fresh buffer solution[15, 17]. The samples were analyzed for drug content by using U.V Visible Spectrophotometer at 258 nm, after appropriate dilutions against isotonic phosphate buffer pH 7.4 as blank.

RESULT AND DISCUSSION:

The efforts were exerted to design and *in-vitro* characterization of Levobunolol HCl occuserts with special reference to glaucoma treatment using polymers like HPMC

(3 & 4 %), PVP (1%) and MC (1 & 2 %). The rate controlling membrane was casted on a glass plate using ethyl cellulose (6%), which is dispersed in chloroform and dibutyl phthalate (30 % w/w of polymer) as plasticizer. The evaluated data of different optimized occusert formulations were shown in **Table-2** and **Fig -1** respectively.

Physical appearance and surface texture:

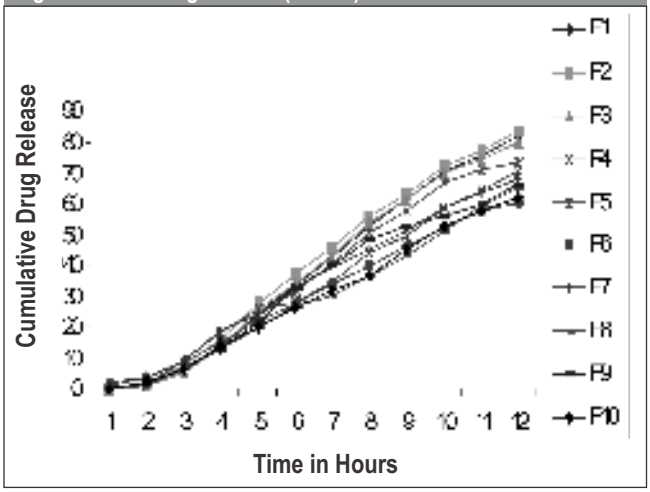
It includes visual inspection as well as texture appearance by feel or touch.

Weight variation:

Weight variation of occuserts was found to be 24.91 (0.09) mg to 34.10 (0.08) mg. The results of weight variation test showed that, there was proper distribution of drug, polymer and plasticizer.

Uniformity of thickness:

Fig. 1: In-Vitro Drug Release (F1-F10)



Thickness of occuserts was measured using screw gauge at five different places and average was taken. It varied between 0.191 (0.002) mm to 0.321 (0.002) mm, which is found to be directly related to the concentration of the polymers.

Tensile strength and % elongation at break:

Tensile strength of occuserts was found to varied with nature of polymer and plasticizer between 0.043 (0.042) to 0.178 (0.075) kg/mm^2. Tensile strength of occuserts can be arranged in order of $F_7>F_9>F_{10}>F_4>F_3>F_8>F_1>F_2>F_6>F_5$. However, % elongation at break showed flexibility of occuserts. It was found to be 21.54 (0.23) to 35.30 (0.51).

% moisture uptake:

Among the formulations tested, F_4 and F_{10} showed maximum and minimum moisture uptake i.e. 14.92 (0.79) and 6.92 (0.07) respectively. The maximum moisture uptake may be due to high concentration of HPMC (Hydrophilic in nature and readily absorbs moisture) when exposed to atmosphere. While minimum moisture uptake was found due to presence of MC (Hydrophobic in nature)

% moisture loss:

Among the formulations tested, F_4 and F_8 showed maximum and minimum moisture loss i.e. 9.92 (0.45) and 4.20 (0.65) respectively. The minimum moisture loss shown by the formulation F_8 was mainly due to EC as rate controlling membrane, which retains the moisture within the matrix.

Folding endurance:

The folding endurance for all formulations was good. Maximum folding endurance was found to be formulation F_4 i.e. 148 (2.2) which is due to presence of PVP. However, minimum folding endurance found to be formulation F_8 i.e. 82 (1.4). This is due to the concentration of MC.

Drug content studies:

Table 2: Evaluation data of different optimized Levobunolol HCl occusert formulations.

FC	Weight Variation (mg)	Thickness (mm)	Tensile Strength (kg/mm^2)	% Elongation at Break	% Moisture Uptake	% Moisture Loss	Folding Endurance	Drug Content (mg)
F_1	29.20 (0.08)	0.272 (0.003)	0.091 (0.031)	21.54 (0.23)	11.53 (0.93)	7.20 (0.28)	94 (1.5)	0.609 (0.003)
F_2	32.15 (0.06)	0.292 (0.004)	0.087 (0.063)	23.12 (0.53)	12.10 (0.68)	7.12 (0.20)	87 (1.7)	0.621 (0.001)
F_3	32.12 (0.05)	0.284 (0.003)	0.129 (0.042)	26.21 (0.25)	13.75 (0.62)	6.71 (0.25)	132 (2.5)	0.592 (0.001)
F_4	34.10 (0.08)	0.321 (0.002)	0.130 (0.033)	28.05 (0.26)	14.92 (0.79)	9.92 (0.45)	148 (2.2)	0.599 (0.002)
F_5	24.91 (0.09)	0.214 (0.002)	0.043 (0.042)	30.28 (0.52)	9.15 (0.65)	8.71 (0.45)	112 (1.2)	0.611 (0.001)
F_6	26.21 (0.07)	0.232 (0.002)	0.053 (0.034)	32.30 (0.35)	8.23 (0.89)	9.20 (0.56)	92 (1.5)	0.611 (0.001)
F_7	27.53 (0.05)	0.243 (0.002)	0.178 (0.075)	35.30 (0.51)	8.01 (0.56)	9.82 (0.25)	114 (1.3)	0.626 (0.002)
F_8	32.20 (0.07)	0.272 (0.003)	0.127 (0.037)	27.25 (0.42)	8.31 (0.52)	4.20 (0.65)	82 (1.4)	0.607 (0.010)
F_9	25.17 (0.08)	0.191 (0.002)	0.148 (0.055)	26.92 (0.34)	7.21 (0.36)	6.23 (0.57)	124 (1.6)	0.603 (0.002)
F_{10}	26.53 (0.02)	0.281 (0.002)	0.139 (0.044)	26.11 (0.21)	6.92 (0.07)	8.92 (0.55)	118 (1.3)	0.616 (0.001)

All values are replicate of five observations;. Figures inside the parenthesis indicate Standard Deviation (S.D) values;. FC indicates Formulation Code.

The drug content was ranging from 0.592 (0.001) to 0.626 (0.002). The estimation of drug content was found to be almost same with their low standard deviation values. Hence, there were no significant variations among the all formulations, which indicate that the method used for preparing occusert was reliable.

***In-vitro* drug release:**

The *in-vitro* drug release studies of all formulations have been shown in **Fig - 1** by plotting graph cumulative % drug release vs time. The results of *in-vitro* drug release studies showed that, formulations (F_2 & F_1) containing 4 % and 3 % HPMC had released their drug content, 83 % and 81 % respectively over an extended period of 12 hr. The extended and prolonged period of drug release may be due to slow diffusion of drug from combined polymers and plasticizers and probably due to formulation of hydrogen bonds between drug and polymers, which have helped in controlling rate of drug release[18]. Therefore, these two formulations were selected as optimized formulations based on there evaluation parameters as well as prolonged drug release study. Based on present study, it can be concluded that, the formulations (F_2 & F_1) of polymeric occuserts containing Levobunolol HCl and plasticizer used with its optimum concentration have considerable influence on their physiochemical characteristics, permeability properties, increased contact time, prolonged drug release and decreased frequency of administration with appreciable strength and safety. Hence, these (F_2 & F_1) formulations were proved to be of great interest and were characterized as best optimized Levobunolol HCl occusert formulations biologically[19].

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