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Research paper

Influence of hydroxypropylmethylcellulose and poloxamer composite on developed ophthalmic *in situ* gel: *Ex vivo* and *in vivo* characterization

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ABSTRACT

The present study was planned to investigate the effect of polymer composites HPMC K15 M and poloxamer 407 on developed dorzolamide hydrochloride *in situ* gel. Formulations were prepared based on 3^2 factorial design with concentrations of Poloxamer 407 and HPMC K15 M as independent variables. Gelation temperature, viscosity study at 37 °C and % cumulative drug release upto 8 h were considered as dependent variables. Polymer composites showed linear model with gelation temperature and quadratic model with viscosity study at 37 °C and % cumulative drug release up to 8 h. Optimized formulation successfully sustained release of drug upto 5 h in *ex vivo* goat corneal permeability study. Comparative *in vivo* study in normotensive rabbits showed that optimized formulation sustained therapeutic effect upto 8 h with 31.22 ± 3.65% reduction in intraocular pressure and marketed formulation showed immediate release effect with 18.22 ± 4.42% reduction in intraocular pressure upto 2–3 h.

1. Introduction

Drug targeting to eye tissue is most challenging area for scientist. Aq's eye drops are the most accepted form of treatment for the administration of ophthalmic drugs in ocular drug delivery systems [1]. Only 1–10% of the drug is absorbed whereas the remaining drug is eliminated from precorneal area [2]. Short residence time, poor bioavailability, poor permeability and rapid precorneal drainage are the major problems for eye drops treatment [3]. Glaucoma is the second leading cause of world's blindness, which may affect around 80 million in 2020 [4]. Glaucoma is complex disease characterized by ocular hypertension with a progressive visual loss that could result in blindness due to damage occurred to the optic nerve. Glaucoma development may be observed due to aging, genetic predisposition, exogenous environmental and endogenous factors [5]. Vision loss occurs in some glaucoma patients due to uncontrollable intra ocular pressure.

Main goal of glaucoma therapy is to reduce increased intraocular pressure in order to prevent deterioration of the optic nerve and loss of vision. The number of drugs and new chemical entities with potential for treating glaucoma is steadily increasing [6]. Topical applied carbonic anhydrase inhibitors (e.g. dorzolamide eye drop solutions) has become one of the most widely used medications for glaucoma treatment [7]. Dorzolamide hydrochloride is twenty times more potent than acetazolamide with regard to inhibition of carbonic acid anhydrase isoenzyme which play major role in aqueous humor secretion [8]. It is also reported that Dorzolamide hydrochloride (Trusopt®; Merck, Whitehouse Station, NJ, USA) and Brinzolamide (Azopt®; Alcon Laboratories, Fort Worth, TX, USA) both have similar IOP–lowering effect [9]. Although dorzolamide hydrochloride is commonly used for glaucoma treatment,

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its conventional eye drops possess poor bioavailability and several adverse effects at high concentration [10]. To overcome the limitations of conventional eye drops it is necessary to develop formulations to prolong the contact time of drug in eye. Literature survey revealed that suspensions, ointments, inserts and aqueous gels are developed for improvement in ocular drug delivery system [11]. These formulations offer some improvement over conventional liquid dosage forms but, have drawbacks of ease of administration, lack of patient compliance, commercial applicability and blurred vision [7]. To overcome these drawbacks *in situ* gel approach has been investigated which offer advantages such as sustained and prolonged action, from a manufacturing point of view the production is less complex and thus lowers the investment and manufacturing costs [12]. Ideal *in situ* forming systems are free-flowing liquid solutions before administration in eye, but became gel under physiological conditions. *In situ* gelling polymers should have pseudoplastic behaviour in tears pH, the temperature of the eye surface or electrolyte present in the tear film [13]. Poloxamer 407 is an insitu gelling agent commonly used in ophthalmic formulations compose of thermo sensitive amphiphillic block copolymers, namely poly (ethylene oxide) – poly (propylene oxide) – poly (ethylene oxide). Poloxamer 407 used in pharmaceutical applications for transdermal, ophthalmic and injectable sustained drug release systems [14–16].

Recently small unilamellar niosomes as ophthalmic carrier of dorzolamide hydrochloride were formulated and *in vitro* studies were carried out to evaluate the formulation but these formulation has limitations like Physical instability, Aggregation, Fusion, Leaking of entrapped drug, Hydrolysis of encapsulated drugs [17,18]. Dorzoalmide hydrochloride loaded chitosan nanoparticles were successfully prepared by ionotropic gelation method [19]. These recently reported studies support the sustained release dorzolamide hydrochloride for the ocular delivery. It will be interesting to mention in this context that the objective of current study attempts has been made to investigate the influence of hydroxypropylmethylcellulose (HPMC K15 M) and poloxamer 407 polymers composite on gelling behaviour of developed dorzolamide hyrochloride *in situ* gel based on 3^2 factorial design and *ex vivo–in vivo* characterization study for confirmation about sustained drug delivery with improved ocular bioavailability effect of developed formulation.

2. Materials and methods

2.1. Materials

Dorzolamide hydrochloride (DZH) was obtained from FDC Ltd (FDC Ltd., Aurangabad, India) and Poloxamer 407 from BASF (BASF Ltd., Mumbai, India). HPMC K15 M was purchased from S. D. Fine Chemicals (S.D. Fine Chemicals, Mumbai, India). All other ingredients and reagents were of analytical grade.

2.2. Methods

2.2.1. Stability indicating RP-HPLC method for DZH

Stability indicating RP-HPLC (Shimadzu LC -20 AD) method for DZH was developed and validated. Drug stability was studied as per International Conference on Harmonization (ICH) guideline Q1A (R2) in recommended acidic, basic, neutral, photolytic, oxidative and thermal stress conditions. The optimized separation was achieved by using a mobile phase consisting of acetonitrile: potassium dihydrogen phosphate buffer (pH 3.5 adjusted by ortho-phosphoric acid) in a composition of 10:90 on Kromasil (250 mm × 4.6, 5 μm) C18 column using isocratic elution program at flow rate was 1 mL/min and was detected 254 nm using a UV detector (SPD-M 20 A, Shimadzu Prominence). Computerized data acquisition and treatment were performed with the LC-solution software.

2.2.2. Gel preparation

Cold method was used to prepare dorzolamide hydrochloride *in situ* gel [20]. Half of the desired volume of distilled water, containing accurately weighed HPMC K15 M, was kept in refrigerator (for cooling) to get a clear solution. The desired amount of Poloxamer 407 was added in HPMC K15 M solution with continuous stirring and solution was stored at 4 °C to obtain clear solution. The solution of desired amount of drug (0.5% w/v), sodium chloride (0.5% w/v) and benzalkonium chloride (0.01% v/v) was prepared in distilled water. This drug, sodium chloride and benzalkonium chloride solution mixed with polymeric solution under constant stirring to get clear solution. The osmolarity of optimized formulation was 298 ± 1.28 mOsmol Kg^{-1}. This value was achieved by the addition of the 0.5% of NACl to the formulation and was determined by using Osmomat 030/050 Terminal model [21].

The volume was made up to 100 ml by purified water. Composition of DZH *in situ* gel is shown in Table 1.

2.2.3. Drug content

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of the formulation with mobile phase and DZH was then determined at 254 nm by using RP-HPLC.

2.2.4. Full factorial experimental design

DZH *in situ* gel optimized by 3^2 randomised full factorial design. Amount of Poloxamer 407 (X_1) and amount of HPMC K15 M (X_2) were considered as independent variables. The actual units of lower, middle and higher levels of factor X_1 were 16%, 18%, 20% and factor X_2 were 0.5%, 0.75%, 1%. The response variables selected gelation temperature (Y_1), viscosity at temperature 37 °C (Y_2) and % cumulative drug release up to 8 h (Y_3).

2.2.5. Clarity and pH

The clarity was determined by using visual inspection under black and white background [22]. The formulation pH was determined by using pH meter (Equiptronics, Model EQ-610).

2.2.6. Measurement of sol-gel transition temperature ($T_{sol-gel}$) and gelation capacity

Test tube containing two ml of *in situ* gel immersed in water bath. The temperature of water bath was increased gradually from room temperature to gel temperature at which gel formed. Gelation temperature was recorded at which gel in the test tube content should not move after tilting it through an angle of 90° whereas gelation capacity was determined by placing formulation drop in vial containing 2 ml artificial tear fluid (pH 7.4) equilibrated at 37 °C, the gel formulation drop was visually evaluated and then gelling capacity recorded [23].

2.2.7. Viscosity study

Viscosity of formulations was measured by using small volume adapter of Brookfield Viscometer (DV-II + Pro, Brookfield Engineering Labs. Inc., Middle boro, USA) equipped with helipath stand and T bar spindle. Viscosity measurements were made at non–physiological (25 °C) and physiological (37 °C) temperature. Measurements were done in triplicate with varying the rotation speed of spindle from 5 to 150 rpm.

2.2.8. In vitro drug release studies

DZH release rates from prepared *in situ* gel were measured across a dialysis membrane using a Franz diffusion apparatus. The