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A novel nanoparticles impregnated ocular insert for enhanced bioavailability to posterior segment of eye: *In vitro*, *in vivo* and stability studies

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ABSTRACT

The present investigation was carried out to demonstrate with the help of *in vitro* and *in vivo* studies that nanoparticles impregnated ocular inserts effectively delivers significant concentration of drug to the posterior segment of eye after topical administration for treatment of glaucoma. Drug loaded Nanoparticles and their ocular insert have been reported to reduce side effects of orally administered Acetazolamide. Eudragit NPs were prepared by the solvent diffusion nanoprecipitation technique. The prepared NPs were evaluated for various parameters such as particle size, zeta potential, % entrapment efficiency, % drug loading, DSC, FTIR, TEM and stability studies. Ocular inserts of NPs were prepared by solvent casting method. The prepared ocular inserts were evaluated for thickness, content uniformity, folding endurance, disintegration time, morphology and stability study. The NPs and ocular inserts were evaluated for *in-vitro* drug diffusion study, *ex-vivo* trans-corneal permeability study, *in-vivo* ocular tolerability and intra ocular pressure (IOP) reduction study. The optimized batch was stable for a period of 3 months in lyophilized form. The optimized formulations had size range of 367 nm ± 8 nm, zeta potential around +7 mV ± 1.3 mV and 51.61% ± 3.84% entrapment efficiency with 19% ± 1.40% drug loading. The *ex-vivo* trans-corneal study showed higher cumulative corneal permeation, flux across corneal tissue (2.460 ± 0.028 μg/ml) and apparent corneal permeability (3.926×10^{-6} cm^2/s & 3.863×10^{-6} cm^2/s) from drug loaded Eudragit NPs and Ocular inserts as compared to drug solution (0.671 ± 0.020 μg/ml & 3.166×10^{-6} cm^2/s). *In-vivo* study showed the Eudragit NPs and ocular insert produced significant ($P < 0.001$) lowering in intra ocular pressure compared with the solution of free drug after 3 h of topical ocular administration. Plain Eudragit NPs caused no inflammation and/or discomfort in rabbit eyes and neither affected the intra ocular pressure establishing their safety and non irritancy.

1. Introduction

New ocular drug delivery systems like nanoemulsion, microemulsion, liposomes, nanoparticles (NPs), microspheres, inserts and collagen shields have been reported since last few years as they can improve pre-corneal residence time and increase ocular bioavailability [1,2]. Some studies on NPs formulation with indomethacin [3], ibuprofen [4], flurbiprofen [5] and ketorolac [6] have shown higher ocular penetration and availability of drug compared to plain drug formulations.

Nanotechnology is a potential approach for ocular delivery of drugs. NPs do not irritate the eye due to their small size. Specially, positive surface charge of NPs can allow a longer residence time to the corneal surface compared to classical eye drops as they interact with negatively charged cornea leading to slower ocular elimination rate, thereby enhancing corneal drug absorption [7–10].

Eudragit RS100 and RL100 are copolymers of poly(ethyl acrylate, methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate) containing quaternary ammonium groups respectively. These polymers are water insoluble and swell irrespective of pH [11].

ACZ, a carbonic anhydrase inhibitor, is used in both closed-angle and open-angle glaucoma for reduction of intra ocular pressure (IOP). Because of its poor aqueous solubility and low permeability coefficient, there is no topical ocular formulation available in market. Only high dose solid oral dosage forms (500–1000 mg/day) and parenteral dosage forms are available which have side effects like metabolic acidosis, renal failure, vomiting, anorexia, diuresis, and central nervous system (CNS) depression [12]. Topical ocular formulations of ACZ like liposomes [13], niosomes [14], nanoemulsions [15], microspheres, surfactant gel

Abbreviations: ACZ, Acetazolamide; IOP, intra ocular pressure; NPs, Nanoparticles; PVA, polyvinyl alcohol; EE, Entrapment efficiency; DR, Drug release; PDI, poly dispersive index.

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[16], microemulsion, cyclodextrin complex [12,17] *etc.* have been reported. However, there is no report of incorporation of ACZ inside polymeric nanoparticulate carriers which can enhance the drug delivery to the ocular surface. Therefore, an attempt was made to prepare and evaluate nanodrug delivery system comprising of ACZ NPs and incorporating them in an ocular insert.

Hence, the aim of the present investigation was to prepare and evaluate Eudragit NPs of ACZ incorporated into an ocular insert for safe and effective topical ophthalmic treatment of glaucoma.

2. Materials

Acetazolamide was kindly gifted by Centaur Pharma, Mumbai, India. Eudragit RS100 was received as a gift sample from Evonik Ltd., Mumbai, India. All other chemicals used in study were of analytical grade (AR). Acetone, methanol, chloroform and polyvinyl alcohol were purchased from S. D. Fine Chemical Limited (Mumbai, India). HPMC (E 15 LV) and propylene glycol were purchased from Loba Chemie Pvt. Ltd., (Gujarat, India).

3. Methods

3.1. Preparation of NPs

Out of many methods for preparation of drug loaded polymeric NPs, the simplest method is the solvent displacement method also known as nanoprecipitation method, developed by Fessi et al. [18]. The method is based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water. The technique is easy, less complex, less energy consuming as well as widely applicable without any additives for the manufacturing of defined nanospheres.

Hence, Eudragit NPs were prepared using solvent diffusion nanoprecipitation method [18]. Organic phase was prepared by dissolving Eudragit in water miscible solvent like acetone. Subsequently, ACZ was added and dissolved in the acetone completely. Aqueous phase comprised of polyvinyl alcohol (medium viscosity, M.W. about 130,000 Da) in distilled water. Organic phase was added drop wise into aqueous phase using syringe (26 gauge) at the rate of 0.1 ml/min with continuous stirring on a magnetic stirrer (Remi Scientific Equipments, Mumbai, India) (700 rpm) at room temperature. Stirring was continued for 8 h to allow evaporation of acetone. Optimization of process variables like concentration of stabilizer, organic:aqueous phase ratio and drug:polymer ratio were carried out by varying one variable at a time and keeping other variables constant as shown in Table 1. All experiment procedures were conducted in triplicate using amber colored apparatus and in dark conditions to protect the drug from light.

Table 1
Formulation variables for ACZ loaded Eudragit NPs.

Batch no.	ACZ Qty. (mg)	Eudragit Qty. (mg)	PVA concentration (%w/v)	Organic phase (ml)	Purified water (ml)
NP-A	35	100	0.0	1.0	5.0
NP-B	35	100	0.5	1.0	5.0
NP-C	35	100	1.0	1.0	5.0
NP-D	35	100	1.0	1.0	5.0
NP-E	35	100	1.0	2.5	5.0
NP-F	35	100	1.0	5.0	5.0
NP-J	10	100	1.0	1.0	5.0
NP-I	15	100	1.0	1.0	5.0
NP-K	20	100	1.0	1.0	5.0
NP-L	25	100	1.0	1.0	5.0
NP-M	30	100	1.0	1.0	5.0
NP-N	35	100	1.0	1.0	5.0
NP-O	40	100	1.0	1.0	5.0

3.2. Preparation of ocular insert

Ocular inserts were prepared by solvent casting method using HPMC E15LV as film forming agent and propylene glycol as plasticizer. HPMC E15LV and propylene glycol were added to the dispersion of ACZ loaded Eudragit NPs (5 ml). The resulting dispersion was stirred using magnetic stirrer at 100 rpm at room temperature for 20 min and thereafter spread on a petridish of 5.0 cm diameter and dried overnight at room temperature for solvent evaporation. The ocular inserts were stored in an air tight container under ambient conditions. Ocular inserts were formulated by the above mentioned method as shown in Table 2.

3.3. Characterization

3.3.1. Characterization of ACZ loaded Eudragit NPs

3.3.1.1. Particle size and zeta potential. The mean particle size of all formulations was determined by photon correlation spectroscopy (PCS) using Malvern Zetasizer Nano ZS (Malvern Instrument Ltd., Worcestershire, United Kingdom). Each sample was appropriately diluted with 0.45 mm-filtered water and the reading was carried out at 90° angle with respect to the incident beam. Zeta potential was calculated by Smoluchowski's equation from the electrophoretic mobility of NPs. All measurements were run in triplicate.

3.3.1.2. % entrapment efficiency and % drug loading. The nanoparticulate suspension was centrifuged (Remi scientific equipment, Mumbai, India) at 3000 rpm for 5 min at room temperature for removal of free drug. The supernatant was then centrifuged at 40,000 rpm for 2 h at 4 °C. The obtained sediment was used for estimation of entrapped drug by dissolving sediment in chloroform: methanol (2:8) and analyzing at 244.0 nm with UV spectrophotometer (Shimadzu-1700, Japan). The amount of drug entrapped in NPs and % drug loading was calculated using the Eqs. (1) and (2) respectively [19]:

$$\%\text{Entrapment Efficiency } (\%EE) = \frac{\text{amount of drug in NPs}}{\text{total amout of drug added}} \times 100 \quad (1)$$

$$\%\text{Drug Loading} = \frac{\text{amount of drug in NPs}}{\text{total amount of drug and polymer used}} \times 100 \quad (2)$$

3.3.1.3. Differential scanning calorimetry (DSC). DSC analysis was carried out using a Differential Scanning Calorimeter (DSC-60, Shimadzu, Japan) at a flow rate of 10 °C/min. DSC thermograms were recorded for ACZ, physical mixture of ACZ with PVA and Eudragit RS 100 (in 1:10:10 ratio), blank Eudragit NPs and ACZ loaded Eudragit NPs.

3.3.1.4. Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectroscopy was used to detect any interactions between ACZ and excipients in the formulation. A Fourier Transform Infrared (FTIR) spectrophotometer (Bruker, Germany) was used. 5 mg of sample was pelleted with dry potassium bromide and the samples were examined at transmission mode over wavenumber range of 4000 to 400 cm^{-1}. FTIR studies were

Table 2
Composition of ACZ loaded Eudragit NPs ocular inserts.

Batch code	PVA (%w/v)	HPMC E15LV concentration (%w/v)	Propylene glycol concentration (%v/w)
OI-1	1%	0.1%	0.1
OI-2	1%	0.2%	0.1
OI-3	1%	0.3%	0.1
OI-4	1%	0.4%	0.1
OI-5	1%	0.5%	0.1
OI-6	1%	0.0%	0.1