SYNOPSIS

1. TITLE

DEVELOPMENT AND CHARACTERIZATION OF NANO-CARRIERS FOR OCULAR DELIVERY OF SOME DRUGS FOR THE EFFECTIVE TREATMENT OF UVEITIS

2. INTRODUCTION

Uveitis is one of the leading causes of blindness that may be associated with systemic inflammation. It causes the inflammation of uvea and neuro-retina which may result in serious loss of vision and morbidity (Foster and Vitale, 2002). Anterior uveitis is a group of inflammatory condition which generally affects the anterior segment, mainly anterior chamber, iris, ciliary body and related surrounding tissue of the eye. In uveitis the primary focus of inflammation is the vitreous, while in posterior uveitis, the retina or choroids are afflicted (Jabs *et al*., 2005).

Uveitis can be categorized as infectious and noninfectious. Intermediate and posterior uveitis may occur as primary ocular process or can be a manifestation of systemic disease. Implications of ocular inflammation include glaucoma and cataract. Posterior segment is also affected and may cause macular edema and vision disorder (Myles *et al.,* 2005). It is reported that 50 % of patients with anterior uveitis have a related systemic illness (Rosenbaun, 1998). Uveitis is a common inflammatory eye disease with reported annual incidences of between 17 and 52.4 per 1,00,000 person-years and prevalence of between 38 and 370 per 1,00,000 population (Dandona *et al.,* 2000; Darrell *et al.,* 1962). Anterior uveitis is the most common form of uveitis in most regions of the world. It accounts for between 50 and 92 % of the total uveitis cases in the Western world and 28-50 % of the cases in India (Chang and Wakefield 2002). Corticosteroids are the mainstay for the treatment of anterior uveitis (Korenfeld, 2008; Noecker and Golightly, 1999). But topical drug delivery is associated with certain prolonged side effects which limit its utility in the management of ocular inflammations. These side effects include decreased immunological response to infection, increased intraocular pressure (IOP), inhibition of re-epithelization, glaucoma and cataract formation (Raizman, 1996; Renfro and Snow, 1992).

The present challenge is to identify novel approaches, for development of safe, effective, nontoxic, long acting and economical drug delivery system for ocular administration. The topical administration has remained the most preferred, convenient and patient – compliant mode of drug administration for treatment of ophthalmic diseases. But ocular bioavailability of drug is compromised due to interplay of several protective mechanisms in the eye. These include the drug absorption barriers, nasolacrymal drainage, binding by lachrymal proteins, limited corneal space. Nanotechnologies represent a promising strategy for improving the ocular delivery of ophthalmic drug. As compared to currently available conventional approaches, these novel topical ocular drug delivery systems can in deliver the drugs at predetermined rate at desired site of action which minimizes the toxic effects with the increase in bioavailability of the drugs. Other benefits include possibility of self-administration, no vision impairment due to small dimensions of delivery systems, protection against metabolic enzymes, possible uptake into deep corneal cells, prolonged drug release, avoid first-pass metabolism reducing drug dose and frequent administration.

In this proposed research work an attempt will be made to develop and characterize novel nanocarrier for topical ocular delivery systems of corticosteroid to increase the precorneal drug residence with efficacy, controlled drug release and achieve higher ocular therapeutic activity for the effective treatment of anterior uveitis.

3. OBJECTIVE OF RESEARCH

The objectives of the proposed research work are:

1) To design polymeric nanocarrier of corticosteroids for ocular administration.
2) To reduce the local and systemic side effects of corticosteroids.
3) To enhance the precorneal retention and prolong the drug release.
4) To compare its efficacy with conventional dosage forms in terms of *in vitro* and *in vivo* performance.
5) To study the stability profile of the developed nanocarrier.

4. BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD

- **Sabzevari et al., (2013)** prepared Poly (D,L-lactide-co- glycolide) (PLGA) polymeric nanoparticle (NP) of triamcinolone acetonide (TA) by using a modified emulsification/solvent diffusion method for the improvement of efficacy in the treatment of uveitis. Anti-inflammatory activity of the prepared formulation was studied in animal model of endotoxin induced uveitis (EIU), and also efficacy was compared with TA injection, TA microparticle and prednisolone acetate (PA). The result indicate that with prepared formulation of PLGA-TA-NP statistically significant difference with the other formulation in terms of providing sustained released, improved ocular bioavailability and can provide better patient compliance was evident.
- **Coffey et al., (2013)** developed a novel ophthalmic gel formulation of loteprednol etabonate (LE), a C-20 ester-based corticosteroid with an established safety profile, in the treatment of ocular inflammatory conditions. The new LE gel formulation was non-settling, eliminating the need to shake the product to resuspend the drug, had a pH close to that of tears, and a low preservative concentration. The rheological characteristics of LE gel were such that it became fluid upon instillation in the eye and retaining sufficient viscosity to prolong ocular surface retention. The new formulation provided consistent, uniform dosing and pharmacokinetic studies in rabbits demonstrated rapid and sustained exposure to LE in ocular tissues. Results from two clinical studies of LE gel in the treatment of postoperative inflammation and pain following cataract surgery indicated that it was safe and effective for ocular therapy.
- **Fitzpatrick et al., (2012)** investigated thermoresponsive resorbable copolymers for minimally invasive drug or cell delivery to the posterior segment of the eye for the treatment of retinal degenerative (RD) disease. The copolymer based on N-isopropylacrylamide (NIPAAm), acrylic acid N-hydroxysuccinimide (NAS) and varying concentrations of acrylic acid (AA) and acryloyloxy dimethyl-γ-butyrolactone (DBA) was fabricated. The result showed that slow-degrading thermoresponsive copolymers are ideal delivery vehicles to provide minimally invasive, sustained, localized release of drug to the posterior segment of the eye.

- **Tommaso et al., (2012)** prepared novel methyl polyethyleneglycol-hexyl substituted polylactides (MPEG-hex PLA) micelle carrier of cyclosporine A (CsA) for topical ocular delivery in the treatment of cornea graft rejection in a rat model after keratoplasty. Confocal analysis of novel formulation with fluorescent labeled micelles confirmed penetration into the all corneal layer. The success rate of cornea graft transplantation was 73% in treated animals against 25% for the control group. This new formulation had the same efficacy like a systemic treatment but without the serious CsA systemic side effects. Ocular drug concentration of transplanted and healthy rat eyes were dosed by UPLC/MS and showed a high CsA value in the cornea (11710 ± 7530 ng_{CsA}/g_{tissue} and 6470 ± 1730 ng_{CsA}/g_{tissue}, respectively). The result revealed that novel micelle formulation can easily penetrate into the deeper layer of ocular tissue and overcome the constraints of ocular barrier. Thus with prolonged CsA release a better alternative to the systemic treatment for the prevention of corneal graft rejection is feasible.
- **Ali et al., (2011)** reported hydrocortisone (HC) nanosuspensions (NS) developed by microfluidic nanoprecipitation method and it was compared with second HC NS prepared by top-down wet milling procedures. Ocular bioavailability of HC nanosuspensions was assessed in albino rabbits using HC solution as a control. A sustained drug action was maintained up to 9 h for the nanosuspensions compared to 5 h for the drug solution. The precipitated and milled NS achieved comparable $AUC_{0–9h}$ values of 28.06 ± 4.08 and 30.95 ± 2.2, respectively, that were significantly ($P < 0.05$) higher than that of HC solution (15.86 ± 2.7). After 2 months storage at room temperature, the milled HC NS showed good stability with no discernable changes in particle size, whereas the particle size of the precipitated HC NS increased to 440 nm.
- **Gan et al., (2010)** reported novel self-assembled liquid crystalline nanoparticles (cubosomes) of dexamethasone coated with Poloxamer 407 produced by fragmenting a cubic crystalline phase of monoolein and water in the presence of stabilizer Poloxamer 407. *In-vivo* pharmacokinetics in aqueous humor was evaluated by microdialysis, which indicated a 1.8-fold (F1) increase in AUC(0->240min) of dexamethasone administered in cubosomes relative to that of Dex-Na phosphate eye drops and the results indicate that formulation improved the preocular retention and ocular bioavailability.

- **Farzaneh et al., (2010)** investigated the *in vivo* effects of a new smart polymer loaded with dexamethasone on endotoxin induced uveitis (EIU) rabbit model. The polymeric micelles were prepared using N-isopropylacrylamide (NIPAAM), vinyl pyrrolidone (VP), and methacrylate (MAA) as monomers in the presence of *N*,*N*-methylene bis-acrylamide (MBA) and triethyleneglycol dimethacrylate (TEGDMA) as cross-linking agents. The result indicate that topical administration of prepared nanosuspensions clearly reduced uveitis symptoms, which were qualified with Hogan scoring. Statistical analysis represented that both the nano formulations significantly reduced inflammation ($p< 0.05$) during 48 hr after endotoxin lipopolysaccharide (LPS) injection. It was concluded that nanosuspension prepared with MBA showed rapid treatment in comparison with other nano formulations. The formulation also showed higher anti-inflammatory activity for a longer duration compared to aqueous suspension of the drug, which is due to small particle size and mucoadhesiveness of polymeric micelles.
- **Zhang et al., (2009**) reported dexamethasone loaded biodegradable PLGA nanoparticles (DEX-NPs) by using solvent evaporation method in rabbits after intravitreal injection. The highest drug loading was obtained using 100 mg PLGA 75:25 in a mixture of acetone-dichloromethane 1:1 (v:v) and 10 mg of dexamethasone. The drug was completely released from this optimized formulation after 4 h of incubation at 37 ◦C. The results demonstrated the feasibility of encapsulating dexamethasone and its subsequent delivery.
- **Nagarwal et al., (2009)** reported nanoparticles of piroxicam using the solvent evaporation / extraction technique, and of methylprednisolone acetate formulated using a copolymer of poly (ethylacrylate, methyl-methacrylate). It was observed that inflammation was inhibited by nanoparticles suspension more efficiently than microsuspension of the drug alone in rabbit eyes with endotoxin induced uveitis (EIU).

- **Barcia et al., (2009)** prepared polylactic-glycolic acid (PLGA) microspheres loaded with dexamethasone to reduce ocular inflammation in rabbits elicited by intravitreal lipopolysaccharide (LPS) injection for short- and long-term ability studies. PLGA microspheres loaded with dexamethasone were prepared by the solvent evaporation technique. The results shows that dexamethasone-loaded microspheres effectively reduced intraocular inflammation caused by LPS in both short- and long-term studies.

- **Fialho et al., (2008**) prepared new vehicle based on a microemulsion for topical ocular administration of dexamethasone using Cremophor EL as surfactant and cosurfactant propylene glycol. The pharmacokinetics of the system was studied in rabbits in order to determine its potential as an absorption promoter, and this was compared to a conventional dosage form. The microemulsion developed produced a higher peak concentration of the drug (1.86 μg/mL) when compared with conventional dosage form.
- **Adibkia et al., (2007a)** developed piroxicam:Eudragi$^{t®}$RS100 nano suspension by using the solvent evaporation/extraction technique to control inflammatory symptoms in the rabbits with endotoxin-induced uveitis (EIU). The *in-vivo* examinations revealed that inflammation inhibition by the drug:polymer nanosuspension was more significant than the microsuspension of drug alone in the rabbits with EIU. The clinical findings reported that the piroxicam:Eudragi$^{t®}$RS100 nanosuspensions may be considered as an improved ocular delivery system for local inhibition of inflammation.
- **Adibkia et al., (2007b)** developed nanoformulations of methylprednisolone acetate (MPA) nanosuspension of a copolymer of poly(ethylacrylate, methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate). Modified quasiemulsion solvent diffusion technique was used for the development of the nanoparticles. The *in vivo* examinations revealed that the endotoxin-induced inflammation can be inhibited by the copolymer nanosuspension more significantly than by the microsuspension of MPA itself in the rabbits with EIU. The result indicated that the copolymer nanosuspension may favor the localized, controlled ocular delivery of MPA for the prevention of inflammatory symptoms in ocular diseases.
- **De Kozak et al., (2004)** reported polyethylene glycol-coated cyanoacrylate nanoparticles loaded with tamoxifen for inhibition of intraocular inflammation in a rat model of EIU. These nanoparticles significantly inhibit the severity and extent of uveitis in treated eyes without any detectable ocular toxicity.

- **Herrero et al., (2001)** developed PLGA microspheres for sustained delivery of dexamethansone to prevent uveitis followed by surgical technique.

- **Vincent et al., (1986)** reported lipid-soluble drug progesterone nanoparticles, coated with Polybutylcyanoacrylate (PBCA) prepared by an emulsion polymerization technique using

a hydrophilic continuous phase. Concentrations of progesterone in various ocular tissues of the albino rabbit were monitored at various times following topical administration of either the nanoparticle suspension or the control solutions. It confirmed the increasing retention efficiency in the precorneal pocket.

5. NOTEWORTHY CONTRIBUTION IN THE FIELD OF PROPOSED WORK

Anterior uveitis is the most common intra ocular inflammation in the anterior segment of the eye. It is a serious vision threatening disorder of the eye. If untreated early it can leads to the development of other disorders of the eye like cataract, glaucoma, increased intraocular pressure (IOP). Presently in the global market the majority of the ophthalmic formulations are eye drops. Although these formulations are convenient, they have the drawbacks like very low ocular drug bioavailability, frequent administration to maintain the drug concentration, naso-lachrymal drainage, systemic adverse effect, and poor patient compliance. Due to the complicated anatomical and physiological barriers of the eye the effective targeted and ocular delivery are difficult to attend. However the potential sight threatening risks of the alternative intravitreal route / periocular injection are retinal detachment, hemorrhage, endophtalmitis and cataract. These are the various issues related with the other alternative ophthalmic route which can be addressed by development of suitable novel nanocarrier drug delivery systems. Application of novel topical ophthalmic nano systems can lead to enhancement of ocular therapeutic activity of corticosteroid by increasing the drug retention and absorption. Implementation of novel nanocarrier ocular delivery technology to corticosteroid molecule may lead to better and desired therapeutic potential as compared to other conventional formulations.

6. PROPOSED METHODOLOGY OF THE RESEARCH WORK

The proposed research work will be carried out according to the following plan:

1. Selection of drug candidate and carrier systems.
2. Procurement of drug, polymer, surfactant, co-surfactant, oils and other consumables.
3. Preformulation studies

(a) Identification of drug by UV & IR spectrophotometry

(b) Solubility in aqueous and non-aqueous solvents and in oils

(c) λ_{max} determination of drug

(d) Partition coefficient studies

(e) Melting point studies

(f) Preparation of standard curve of drug

(g) Assessment of drug excipient compatibility by FTIR / DSC / XRD.

5. Development and optimization of novel nanocarriers of selected drug for ocular delivery.

6. Characterization and evaluation of the nanosystems

(a) Shape and morphology by electron microscopy (SEM / TEM)

(b) Average particle / droplet size and Polydispersity index by Photon correlation spectroscopy (PCS)

(c) Zeta potential

(d) Percentage yield

(e) Entrapment efficiency and drug content

(f) *In-vitro* drug release studies.

7. Preparation and evaluation of nanocarrier system loaded formulation

(a) Assessment of Physical attributes

(b) *In-vitro* drug release study.

8. In-vivo studies

(a) Ocular irritation study

(b) Drug retention study

(c) Anti-inflammatory activity

9. Stability studies as per ICH guidelines

10. Patent filing

11. Compilation and presentation of data

7. EXPECTED OUTCOME OF THE PROPOSED WORK

The objective of proposed research work is to develop and characterize of corticosteroid loaded novel biodegradable nanocarrier for ocular administration. The nanocarrier is anticipated to provide potential benefits in prolonging drug precorneal retention, high ocular bioavailability, bypass the systemic drug absorption and toxicity and reduced the dosing frequency thus achieve the desired ophthalmic therapeutic activity along with improved patient compliance. It is expected that the proposed nanotechnology based formulation should deliver the drug to the site of action and control the vision threatening ocular disorder of anterior uveitis.

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Signature of Supervisor	Signature of Co-supervisor	Signature of Candidate
(Dr. (Mrs.) Preeti K. Suresh)	(Dr. S. J. Daharwal)	(Abhishek Kumar Sah)

Forwarded

Chairman, DRC
University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.)