

1 INTRODUCTION

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The diseases of eye can vary from superficial eye diseases, such as infections (e.g., conjunctivitis, blepharitis, keratitis sicca) to the diseases of the posterior segment like cytomegalovirus (CMV) retinitis, uveitis, diabetic retinopathy, retinal degeneration, glaucoma, age related macular degeneration etc., which cause vision impairment and blindness (Arujo et al., 2009; Hughes et al., 2005). Moreover, the eye presents unique challenges when it comes to the efficient delivery of pharmaceuticals to the different sections of eye through topical route as every ocular tissue have its own permeability and solubility characteristics (Pandit et al., 2009). Generally, for treating diseases of the anterior segment topical route is the most preferred route but due to various challenging constraints like lacrimal and nasolacrimal drainage, drug binding and metabolism by tear proteins, target non-specificity resulting from systemic absorption through the nasal and lacrimal duct mucosa and conjunctival vasculature, topically applied drugs are rapidly eliminated from the ocular surface (Stjernschantz et al., 1993; Patton et al., 1978; Frishman et al., 2001).

As compared to anterior segment, treatment of the posterior segment diseases viz. vitreous, retina and choroid are required to be treated with either systemic administration or through intravitreal injections and vitreal implants. While therapy with systemic administration requires large doses due to strong blood-ocular tissue barrier, the other two routes are very invasive, requiring skilled administration, and are associated with a high degree of risk, such as development of retinal detachment and endophthalmitis (Pandit et al., 2009).

Among such diseases, Cytomegalovirus (CMV) retinitis is the most dreadful intraocular infection that affects approximately 85% of persons with HIV whose CD4⁺ T cell count reaches below 50 cells/ μ l (Deayton et al., 2004). CMV retinitis is caused by cytomegalovirus (CMV), which is a double-stranded DNA virus of approximately 220 kb and is a member of the beta class of human herpesviruses. Cytomegalovirus can easily be transmitted through contact with bodily fluids or by placental transfer (Pass et al.,

1985). This infection mainly affects endothelial cells of ocular vessels, optic nerve and the retina, resulting in direct or autoimmune damages like 'uveoretinitis' and disturbed vision. Ganciclovir is the drug approved for treating CMV (Rahi et al., 1984). Ganciclovir (GCV) exhibits antiviral activity against herpes simplex virus (HSV) and cytomegalovirus (CMV) at relatively low inhibitory concentrations (IC_{50} of ~50 and 900 ng/mL, respectively) (Mar et al., 1983; Martin et al., 1983; Cantrill et al., 1989; Markham et al., 1984).

Currently, systemic route & invasive methods are used for delivery of GCV to treat CMV retinitis. In most cases, an intravenous dose (10 mg/kg daily, 7 to 21 days) of ganciclovir halts disease progression. Unfortunately, the disease recurs after discontinuation of the drug. Even on maintenance therapy, CMV recurs in 30 to 50% of patients. Dose-dependent myelosuppression prevents maintenance therapy in about 15% of patients. Sepsis related to permanent indwelling catheters is another problem associated with systemic ganciclovir administration (Herrero-Vanrell et al., 1998). Intravitreal ganciclovir injection is another option, though frequent injections are required and the fellow eye and distant organs are not protected. Standard doses range from 200 μ g to 400 μ g administered twice a week, for up to 3 weeks, followed by weekly maintenance injections (Lima et al., 2004). These intravitreal injection causes increased risk of complications like endophthalmitis, retinal detachment, vitreous hemorrhage, optic atrophy, keratitis, and subconjunctival hemorrhage (Yasukawa et al, 2004). The ganciclovir implant (Vitrasert) prevents progression of CMV retinitis, but requires surgery and may be associated with an increase in early retinal detachment (Daniel et al, 2004).

Another dreadful disease that causes 10% of visual losses and 5–20% of cases of blindness in developed countries is Uveitis (Mishima et al, 1981). Uveitis includes ocular autoimmune or inflammatory diseases involving the iris, the ciliary body, the choroid, and/or adjacent tissues (Yasukawa et al, 2004). Uveitis is a general term used to describe inflammation of the uveal tract, which is the middle layer of the eye, between the sclera, conjunctiva and the anterior chamber on the outside and the retina

on the inside (Munoz-Fernandez et al., 2006). It has acute or chronic features covering a local or diffuse area, and it has the potential to recur (Yasukawa et al, 2004).

The disease often requires long-term pharmacologic therapy with steroids, immunosuppressive agents, antibiotics, or all of these to suppress chronic inflammation or prevent recurrence in specific cases (Munoz-Fernandez et al., 2006). Triamcinolone acetonide (TA) is a relatively safe and effective agent for treatment of conditions requiring long term ocular steroid administration such as uveitis, macular edema secondary to retinal vascular disease, and intraocular proliferations such as choroidal neovascularization (CNV) in age-related macular degeneration (ARMD) and vitreoretinopathy (Shell, 1982). However, while the drug is effective, the drug delivery system is not ideal. Complications of intravitreal TA therapy include secondary ocular hypertension, cataractogenesis, postoperative infectious and non-infectious endophthalmitis and pseudo-endophthalmitis. Besides, any intraocular injection carries the risk of vision loss from infection, retinal detachment or haemorrhage (Schoenwald, 1990). Recently, vitrectomy was reported to be effective in the treatment of uveitis in the posterior segment (Scott et al., 2003). Because the device for drug-controlled release was biocompatible in patients with CMV retinitis (Morley et al., 1995; Sanborn et al., 1992; Smith et al., 1992), uveitis is currently being targeted with the same type of device that releases corticosteroids (Jaffe et al., 2000 a, b).

Thus, there is a pressing need for non invasive and harmless delivery systems targeting the posterior segment of the eye for the treatment of these diseases. The possibility of reaching the posterior segments of the eye through topical instillation of formulations is of great clinical interest as it provides various advantages for drug administration, including direct and localized delivery to the target tissue, better accessibility into the intraocular environment than can generally be achieved by systemic delivery, convenience, and relative painlessness (Davis, 2000; Maurice, 2002; Takashaki et al., 2003). However, this required specialized drug delivery systems, as conventional formulations like eye drops are unable to achieve therapeutic drug levels in the retina because of the presence of corneal and conjunctival epithelia and tear film which

serves as biological barriers to protect the eye from potentially harmful substances and drugs (Inokuchi et al., 2010).

The use of colloidal nanoparticulate drug delivery systems can be exciting new modalities of drug delivery that offer effective treatment of visually devastating diseases and as a way to enhance the bioavailability of drugs administered both systemically and topically (Dayle et al., 2000). Nanoparticulate drug delivery systems include nanorange carriers (100-1000 nm) like nanoparticles, nanospheres, nanoemulsion, polymeric micelles, nanogels etc. The biologically active agent can be dissolved or encapsulated in the macromolecular material composing the particles (Dobrovolskaia et al., 2008).

Ophthalmic drug delivery, more than any other route of administration, may benefit to a full extent from the characteristics of nanoparticulate systems (Tamilvanan et al., 2004). The nano-size of nanoparticles provides smaller particle size resulting in higher surface area available for mucoadhesion which ultimately leads to increased bioavailability and corneal penetration. It has been recommended that particles should be less than 10 μm to minimize particle irritation to the eye, decrease tearing and drainage of instilled dose and therefore increase the efficacy of an ocular treatment (Yasukawa et al., 2004).

The use of nanoparticles as drug carriers for ocular drug administration is an interesting approach; nanoparticulate systems have a smaller size compared to microparticles; as a result they diffuse rapidly and are internalized in the ocular tissues and cells of the anterior and posterior segments (Merodio et al., 2002). Therefore, these carriers may contribute to the preparation of a more efficacious and secure pharmaceutical dosage form which may improve the patient acceptance and compliance (Hosoya et al., 2005).

➤ **Emulsomes:**

Emulsomes are novel lipoidal vesicular systems with an internal solid fat core surrounded by a phospholipid bilayer. This drug delivery carrier has features intermediate between liposomes and oil-in-water emulsions. Emulsomes have a lipid

assembly consisting of a hydrophobic core as can be found in oil-in-water emulsions. This core is surrounded and stabilized by one or more phospholipid layers, which are also principal components of liposomes. The characteristic feature of emulsomes is that the core is composed of lipid which at 25 °C in bulk form, should be in solid or liquid crystalline phase, rather than an oil in a fluid phase. These features make emulsomes more stable than liposomes and liquid emulsion, which could be of considerable advantage in clinical practice. Moreover, as these lipid carriers have the characteristics of both liposomes and emulsions, they can be useful in high hydrophobic drug loading in the internal solid lipid core and the ability to encapsulate water-soluble medicaments in the aqueous compartments of surrounding phospholipid layers (Lowell et al., 1997).

In addition to vehicles for parenteral drug delivery, emulsomes can be used for instillation into the eye, topical delivery to the lungs as aerosols or nebulae, topical delivery to the skin as a dermatological ointment, intranasal administration as droplets, and oral or rectal administration (Amelem et al., 1984).

➤ **Chitosan Nanoparticles:**

Among the various bioadhesive materials that have been proposed for ocular delivery of drugs, chitosan, a copolymer of glucosamine and N-acetyl glucosamine, has received particular interest. Chitosan has been studied as a biomaterial and as a pharmaceutical excipient for drug delivery, because of its favorable biological properties (Lee et al., 1995). It is biocompatible with living tissues since it does not cause allergic reactions and rejection. It breaks down slowly to harmless products (amino sugars), which are completely absorbed by the human body (Nicol et al., 1991). Chitosan degrades under the action of ferments; it is nontoxic and easily removable from the organism without causing concurrent side reactions. It possesses antimicrobial property and absorbs toxic metals like mercury, cadmium, lead, etc. In addition, it has good adhesion, coagulation ability, and immune-stimulating activity (Aria et al., 1968).

In spite of its reported successes, a major drawback of chitosan is that it is insoluble at physiological pH, whereas it is soluble and active as an absorption enhancer only in its protonated form in acidic environments. This is because chitosan is a cationic polysaccharide and in neutral or basic pH conditions it contains free amino groups and hence, is insoluble in water. In acidic pH, amino groups can undergo protonation thus, making it soluble in water. Solubility of CS depends upon the distribution of free amino and N-acetyl groups. Usually 1–3% aqueous acetic acid solutions are used to solubilize CS (Sannan et al., 1976).

In contrast, Chitosan hydrochloride salt (CS HCl), a partially quaternized chitosan derivative, shows good water solubility and has an added advantage compared to chitosan base because it is soluble in water at neutral pH. Chitosan has recently been approved and a monograph relating to chitosan hydrochloride was included in the fourth edition of the European Pharmacopeia (2002). This chitosan salt extracted from crustaceans' shells must have a deacetylation degree of 70–95% (Panos et al., 2008).

Various water soluble chitosan derivatives have numerous advantageous features in comparison to chitosan, such as significantly improved mucoadhesive and enhanced penetration of drugs and peptides through the mucosa by opening the tight junctions between epithelial cells or by intracellular routes. The strong cohesive properties of modified chitosans make them highly suitable excipients for controlled drug release dosage forms (Rossi et al., 2001; Alonso and Sanchez, 2003; Felt et al., 2000; Artursson et al., 1994; Dodane et al., 1999).

Chitosan has many advantages, particularly for developing micro/nanoparticles. If degree of deacetylation and molecular weight of CS can be controlled, then it would be a material of choice for developing micro/nanoparticles. Another potential advantage of chitosan nanoparticles is that they can be rapidly fabricated under extremely mild conditions and can incorporate bioactive compounds (Calvo et al., 1997).

Another interesting aspect of chitosan is that different biomaterials can be used with chitosan which may lead to the development of interesting carriers with new and improved properties. Among them, chitosan / hyaluronic acid nanoparticles and a new type of nanosystems that combine the positive features of polysaccharides with those of lipids to form lecithin/ chitosan self organized nanoparticles.

1.2 AIMS AND OBJECTIVES

The aim of the study was to develop and evaluate the potential of nanoparticulate carriers like emulsomes and nanoparticles for ganciclovir and triamcinolone acetonide, to reach the posterior segment of eye via topical route with the following objectives:

- To reduce the complications, inconvenience and side effects of invasive surgical methods and systemic route, currently used for the delivery of drugs into the posterior segment of eye.
- Ease of drug application & increased patient compliance.

1.3 PLAN OF WORK

Ganciclovir and Triamcinolone acetonide loaded emulsomes and chitosan nanoparticles were prepared with following plan of work:

- i) Literature survey, procurement of active pharmaceutical ingredients and excipients.
- ii) Preformulation studies and development of analytical methods for active pharmaceutical ingredients.
- iii) To prepare water soluble chitosan hydrochloride salt from medium molecular weight chitosan.
- iv) Formulation development of ganciclovir and triamcinolone acetonide loaded emulsomes and chitosan hydrochloride nanoparticles.
- v) Optimization of ganciclovir and triamcinolone acetonide loaded emulsomes and chitosan hydrochloride nanoparticles by applying factorial designs.
- vi) To characterize the prepared formulations for particle size, zeta potential, % drug entrapment efficiency.
- vii) To determine the *in vitro* release properties of drug loaded formulations and plain drug.

- viii) To determine the corneal permeation of drugs loaded formulations using goat cornea model.
- ix) To establish the stability profile of drugs loaded formulations.
- x) To perform the short term exposure test on SIRC cell lines in order to evaluate the *in vitro* irritation potential of excipients used in the formulations.
- xi) To perform cytotoxicity studies on the formulations (24 hrs) in SIRC cell lines to evaluate the effect of different concentration of formulations on % viability of cells.
- xii) To determine the *in vivo* performance of formulations by performing precorneal retention studies in rabbits and ocular distribution studies in mice.



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