1 LITERATURE REVIEW

1.1 ANATOMY AND PHYSIOLOGY OF EYE

The unique anatomy, physiology and biochemistry of eye render this organ impervious to foreign substances, thus presenting a constant challenge to the formulator to circumvent the protective barriers of the eye without causing permanent tissue damage (Karthikayen et al., 2008).

1.1.1 Structure of the ocular globe

Anatomically, the eye is divided into the anterior and posterior segments. The former includes the cornea, the anterior chamber, the iris, the ciliary body, and the lens; the latter is composed of the vitreous, the choroid, the sclera, the retina and the retinal pigment epithelium (Ambati et al., 2002).

Fig 1.1 Schematic representation of the structure of the human eye (Ludwig et al., 2005)

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1.1.1.1 Anterior Segment

The eyelids are under involuntary (spontaneous or periodic blinking and reflex blinking) and voluntary control. They distribute the tear fluid over the eye, providing an optically smooth surface over the cornea (Robinson, 1993).

Cornea: Cornea is a clear transparent epithelial membrane. Light rays pass through the cornea to reach the retina. The cornea is convex anteriorly and is involved in refracting (bending) light rays to focus them on the retina. It is composed of five layers: epithelium, Bowman’s layer, stroma, Descemet’s membrane and endothelium (Fig 2.1) (Greaves et al., 1983; Robinson, 1993).

The conjunctiva is a thin transparent membrane, which lines the inner surface of the eyelids and is reflected onto the globe. At the corneal margin, it is structurally continuous with the corneal epithelium. The membrane is vascular and moistened by the tear film (Greaves et al., 1983; Robinson, 1993). The conjunctiva is composed of an epithelium, a highly vascularised substantia propria, and a submucosa or episclera. The bulbar epithelium consists of 5 to 7 cell layers. The structure resembles a palisade and not a pavement when compared to the corneal epithelium. At the surface, epithelial cells are connected by tight junctions, which render the conjunctiva relatively impermeable. The conjunctival tissue is permeable to molecules up to 20,000 Da, whereas the cornea is impermeable to molecules larger than 5000 Da (Greaves et al., 1983; Huang et al., 1989).

Aqueous Humour: It is a jelly-like substance located in the anterior chamber of the eye (Kumar et al., 2011).

- Iris: The iris is the visible coloured part of the eye and extends anteriorly from the ciliary body, lying behind the cornea and in front of the lens. It divides the anterior segment of the eye into anterior and posterior chambers which contain aqueous fluid secreted by the ciliary body.

- Pupil: The pupil is the aperture through which light – and hence the images we see and "perceive" - enters the eye.
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- **Lens:** The lens of the eye is a flexible unit that consists of layers of tissue enclosed in a tough capsule. It is suspended from the ciliary muscles by the zonule fibers.

- **Ciliary Muscle:** The ciliary muscle is a ring-shaped muscle attached to the iris. It is important because contraction and relaxation of the ciliary muscle controls the shape of the lens (Kumar et al., 2011).

1.1.1.2 Posterior Segment

Posteriorly, the eyeball has a wall consisting of three layers: the outer coat or the sclera, a middle layer or uveal coat and the inner coat or retina (Fig 2.1).

- **Sclera:** The sclera is a tough white sheath around the outside of the eye-ball. It consists of a membrane that maintains the shape of the eye and gives the attachment to the extrinsic muscle of the eye.

- **Uveal tract** is the middle layer of the eye, between the sclera, conjunctiva and the anterior chamber on the outside and the retina on the inside.

- **Choroid:** The choroid layer is located behind the retina and absorbs unused radiation.

- **Retina:** The retina may be described as the "screen" on which an image is formed by light that has passed into the eye via the cornea, aqueous humour, pupil, lens, then the hyaloid and finally the vitreous humour before reaching the retina.

- **Vitreous Humour:** The vitreous humour (vitreous body) is a jelly-like substance.

- **Optic Nerve:** The optic nerve is the second cranial nerve and is responsible for vision. Each nerve contains approximately one million fibres transmitting information from the rod and cone cells of the retina (Kumar et al., 2011).

1.2 BARRIERS OF THE EYE AND ROUTES OF ADMINISTRATION

The eye is partially isolated from the remainder of the body by several types of barriers that impede the effective passage of many drugs (Fig 2.2), leading to minimal dose absorption (Yasukawa et al., 2004)

Most of these are anatomical and physiological barriers that normally protect the eye from toxicants. In general, these barriers consist of the (i) muco-aqueous layer of the...
tear film that protects the anterior surface of the eye, (ii) corneal epithelium with abundant tight junctions and desmosomes, (iii) iris blood vessels that lack fenestrations, (iv) non-pigmented layer of the ciliary epithelium that constitutes the blood aqueous barrier and limits the passage of molecules from the blood to the inner part of the eye, and (v) retinal pigment epithelium (RPE), along with the endothelium of the retinal vessels, constitute the inner and the outer blood retina barriers, respectively, that limit the passage of molecules from the blood to the retina and vitreous cavity (Urtti, 2006). These barriers are specific depending upon the route of administration viz. topical, systemic, and injectable. Moreover, various preformulation and formulation factors need to be considered while designing an ophthalmic formulation.

![Fig 1.2 Routes of drug administration to eye (Gaudana et al., 2010)](image)

**1.2.1 Topical administration**

Topical administration, mostly in the form of eye drops, is employed to treat anterior segment diseases. For most of the topically applied drugs, the site of action is usually different layers of the cornea, conjunctiva, sclera, and the other tissues of the anterior segment such as the iris and ciliary body (anterior uvea). Upon administration, precorneal factors and anatomical barriers negatively affect the bioavailability of topical
formulations. Precorneal factors include solution drainage, blinking, tear film, tear turn over, and induced lacrimation (Ananthula et al., 2009).

In addition, various layers of the cornea, conjunctiva, and sclera play an important role in drug permeation. The cornea, the anterior most layer of the eye, is a mechanical barrier which limits the entry of exogenous substances into the eye and protects the ocular tissues.

Compared to cornea, conjunctival drug absorption is considered to be nonproductive due to the presence of conjunctival blood capillaries and lymphatics, which can cause significant drug loss into the systemic circulation thereby lowering ocular bioavailability. Conjunctival epithelial tight junctions can further retard passive movement of hydrophilic molecules (Saha et al., 1996).

The sclera, which is continuous with the cornea originates from the limbus and extends posteriorly throughout the remainder of the globe. The sclera mainly consists of collagen fibers and proteoglycans embedded in an extracellular matrix. Permeability through the sclera is considered to be comparable to that of the corneal stroma. Recent reports indicate that the permeability of drug molecules across the sclera is inversely proportional to the molecular radius.

1.2.2 Systemic (parenteral) administration

Following systemic administration, the blood–aqueous barrier and blood–retinal barrier are the major barriers for anterior segment and posterior segment ocular drug delivery, respectively. Blood–aqueous barrier consists of two discrete cell layers located in the anterior segment of the eye viz. the endothelium of the iris/ciliary blood vessels and the nonpigmented ciliary epithelium. Both cell layers express tight junctional complexes and prevent the entry of solutes into the intraocular environment (Barar et al., 2008) such as the aqueous humor. However, outer blood–retinal barrier (RPE) restricts further entry of drugs from the choroid into the retina. Even though it is ideal to deliver the drug to the retina via systemic administration, it is still a challenge due to the blood–retinal barrier, which strictly regulates drug permeation from blood to the retina. Hence, specific oral or intravenous targeting systems are needed to transport molecules through the choroid into deeper layers of the retina (Urtti et al., 2006).

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1.2.3 Oral administration
Oral delivery (Santulli et al., 2008; Shirasaki et al., 2006; Kampougeris et al., 2005) or in combination with topical delivery (Sakamoto et al., 2008) has been investigated for different reasons. Topical delivery alone failed to produce therapeutic concentrations in the posterior segment.

1.2.4 Periocular and intravitreal administration
Although not very patient compliant, these routes are employed partly to overcome the inefficiency of topical and systemic dosing to deliver therapeutic drug concentrations to the posterior segment. Moreover, systemic administration may lead to side effects making it a less desirable delivery route for geriatric patients. The periocular route includes subconjunctival, subtenon, retrobulbar, and peribulbar administration and is comparatively less invasive than intravitreal route (Fig 2.3).

![Fig 1.3 Schematic representation of periocular routes of drug administration (Thirmawithana et al., 2011)](image)

The posterior elimination pathway involves drug permeation across the blood-retinal barrier and requires optimum passive permeability or active transport mechanisms. As
a result, hydrophilicity and large molecular weight tend to increase the half-life of the compounds in the vitreous humor (Urtti, 2006).

1.3 DISEASES OF POSTERIOR SEGMENT OF EYE AND THEIR TREATMENT STRATEGY

As compared to anterior segment, it is much harder to deliver drugs to the posterior segment because of the longer diffusional distance and counter directional intraocular convection from the ciliary body to Schlemm’s canal (Yasukawa et al., 2004). Moreover, diseases of the posterior segment of the eye effecting the posterior sclera, the uveal tract, vitreous, choroid, retina and optic nerve head (ONH) like CMV retinitis, uveitis, diabetic retinopathy, retinal degeneration, glaucoma, age related macular degeneration etc., are becoming increasingly amenable to pharmacotherapy as these diseases cause impaired vision and blindness for millions of patients around the world (Karthikayen et al., 2008)

1.3.1 Cytomegalovirus retinitis

Among these 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/μl (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 (IC₅₀ 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 µg to 400 µg 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).

1.3.2 Uveitis
Uveitis is the cause of 10% of visual losses and 5–20% of cases of blindness in developed countries (Mishima et al., 1981). Uveitis includes ocular autoimmune or inflammatory diseases involving the iris, the ciliary body, the choroid, and/or adjacent tissues. 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. It has acute or chronic features covering a local or diffuse area, and it has the potential to recur. The International Uveitis Study Group classification system categorizes uveitis as follows:

- ‘anterior’ when it involves the iris or the ciliary body (iritis of iridocyclitis);
- ‘posterior’ when it affects the choroid or, by extension, the retina (choroiditis or retinochoroiditis). Posterior uveitis may or may not develop into retinal vasculitis
- ‘intermediate’ when the inflammation is limited to the vitreous, peripheral retina, pars plana or the ciliary body; or
- ‘panuveitis’ when two or more of these segments are affected.

The lengthy period of inflammation in the anterior segment sometimes results in rigidity of the iris and secondary glaucoma and cataract, while inflammation in the...
posterior segment sometimes leads to hazy vitreous, macular edema, and other retinal dysfunctions. 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.

Current treatment for uveitis employs systemic medications that have severe side effects and are globally immunosuppressive. Clinically, chronic progressive or relapsing forms of non-infectious uveitis are treated with topical and/or systemic corticosteroids. In addition to steroids, macrolides such as cyclosporine and rapamycin are used, and in some cases cytotoxic agents such as cyclophosphamide and chlorambucil, and antimetabolites such as azathioprine, methotrexate, and lefunomide. While often effective, these drugs have potential serious side effects and they compromise protective immunity to pathogens (Nussenblatt et al., 1996). Recently, vitrectomy was reported 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., 2000a, b).

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). Intravitreal injection of triamcinolone acetonide (IVTA) in human ophthalmology is an increasingly common treatment for a variety of ophthalmic conditions (Domínguez and Quiroga, 2004; Gillies et al., 2004; Jonas et al., 2005; Velez and Whitcup, 1999). Intravitreally injected triamcinolone acetonide bypasses the blood-ocular barrier allowing higher intraocular drug concentrations than can be achieved by systemic or topical administration. It also provides relatively constant drug levels; hence much less drug is needed (Velez and Whitcup, 1999). Triamcinolone crystals have been observed for up to 3 months following 4 mg IVTA.
(Beer et al., 2003), and mass spectrometry has detected low concentrations of these crystals for up to 18 months following 20–25 mg IVTA (Jonas, 2004).

However, the drug is effective, the drug delivery route is not ideal as a result various complications exist from intravitreal TA therapy which majorly includes secondary ocular hypertension, cataractogenesis, postoperative infectious and non-infectious endophthalmitis and pseudo-endophthalmitis, increased intraocular pressure (IOP), sub-conjunctival haemorrhage, reflux of vitreous-like fluid and subcapsular cataract. Besides, this intraocular injection also carries the risk of vision loss from infection, retinal detachment or haemorrhage (Schoenwald, 1990; Gupta et al., 2005; Jonas et al., 2004; Jost et al., 2004; Ciardella et al., 2004; Jonas et al., 2005).

1.4 TOPICAL OCULAR DELIVERY TO THE POSTERIOR SEGMENT

1.4.1 Constraints to topical posterior segment delivery

A fundamental mechanistic understanding of the absorption, distribution, and elimination pathways for delivery of drugs to the posterior segment are required for rational treatment paradigms. The eye poses quite novel and unique constraints to retinal drug delivery. Schematic presentation of the ocular structure with the routes of drug kinetics is illustrated in Fig 2.4

Fig 1.4 Schematic presentation of the ocular structure with the routes of drug kinetics illustrated.
The numbers refer to following processes: (1) trans-corneal permeation from the lacrimal fluid into the anterior chamber, (2) non-corneal drug permeation across the conjunctiva and sclera into the anterior uvea, (3) drug distribution from the blood stream via blood–aqueous barrier into the anterior chamber, (4) elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and Sclemm's canal, (5) drug elimination from the aqueous humor into the systemic uveoscleral circulation, (6) drug distribution from the blood into the posterior eye across the blood–retina barrier, (7) intravitreal drug administration, (8) drug elimination from the vitreous via posterior route across the blood retina barrier, and (9) drug elimination from the vitreous via anterior route to the posterior chamber (Urtti, 2006).

Topical instillation of ophthalmic drops is clearly the most common method used to administer treatments for ocular disease. However, the anatomy and physiology of the eye pose almost insurmountable barriers for delivery to the posterior structures. Productive absorption from topical delivery has been described as occurring by two routes: the corneal and non-corneal (conjunctival/scleral) pathways (Fig. 5). The barriers to productive topical absorption of drugs into the anterior chamber are well documented.

The non-corneal or conjunctival/scleral route of absorption involves penetration across the conjunctiva and sclera and then into the intraocular tissues. This mechanism of absorption was once thought to be nonproductive. However, many studies have shown that the conjunctival/scleral route is significant for compounds with poor corneal permeability such as inulin, timolol maleate, gentamicin, bimatoprost and prostaglandin PGF2-ALPHA (Schoenwald et al., 1997; Bito et al., 1981; Ahmed et al., 1985; Bloomfield et al., 1978).

Penetration and distribution of a drug into the posterior tissues of the eye after topical administration can occur by the following pathways:

1. Drug can diffuse into the iris root and subsequently into the posterior chamber aqueous humor and into the posterior tissues.
2. Drug can enter directly through the pars plana without encountering the blood–
retinal barrier.
3. Drug can diffuse across the sclera by lateral diffusion followed by penetration of
Bruch’s membrane and the RPE.
4. To a lesser extent, drug can be absorbed into the systemic circulation either through
the conjunctival vessels or via nasolacrimal duct and gain systemic access to the
retinal vessels.

Fig 1.5 Drug distribution pathways through the corneal and conjunctival/sclera routes following
topical administration (Hughes et al., 2005)

Compounds diffusing into the vitreous from the posterior chamber will develop a
collection gradient extending from the aqueous humor into the vitreous. This
collection gradient, however, is shallow and rapidly reversed as the aqueous humor
collection of drug falls (Maurice et al., 1986). This can result in a very low
collection concentration of drug in the peripheral retina and anterior vitreous and an extremely
low concentration in the posterior vitreous and fovea. These pathways are not mutually
exclusive and have one common feature; they all involve drug penetration through the
conjunctiva following topical administration. Variables that may determine the

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contribution of each pathway include the rate-limiting barriers to the target site and the chemical characteristics of the drug. Enhancing the permeation of drugs through the rate-limiting barriers, the conjunctiva, RPE and even the sclera, would be expected to benefit posterior penetration.

At first glance, the sclera appears a relatively uniform tissue composed of a large amount of type I collagen, a few elastic fibers, and some fibroblasts. The proteoglycans, making up most of the amorphous gel in the interfibrillar space, have been thought to be chiefly responsible for the diffusional pathway for hydrophilic drugs (Maurice et al., 1986; Geroski et al., 2001; Ambati et al., 2002; Yasukawa et al., 2004; Palestine et al., 1981).

Some lipophilic drugs may passively penetrate the RPE. Hence, a few compounds may directly access the posterior segment by lateral scleral diffusion followed by penetration of Bruch's membrane and the RPE. Unfortunately, the choroidal blood flow is anterior and the choriocapillaris fenestrated leading to rapid removal of compounds from the sclera. For significant levels in the posterior segment to be achieved the drug must have a high permeability to the RPE, have some depot effect and have a sustained concentration gradient to drive the process. The high extent of binding of beta blockers by Tenon's capsule following long term treatment with betaxolol and timolol suggests that this mechanism might provide prolongation of drug delivery (Sponsel et al., 1999).

1.5 **NANOPARTICULATE DRUG DELIVERY**

Nanotechnology, a term derived from the Greek word nano, meaning dwarf, applies the principles of engineering, electronics, physical and material science and manufacturing at a molecular or submicron level. Materials at the nanoscale could be a device or a system or, alternatively, supramolecular structures, complexes or composites. An early promoter of nanotechnology, Albert Franks, defined it as that area of science and technology where dimensions and tolerances are in the range of 0.1–100 nm (Sahoo et al., 2003).
Various criteria that are needed to be considered, while deciding the formulation parameters for developing a suitable ophthalmic drug delivery system are shown in Table 2.1.

**Table 2.1 Criteria for the selection of optimal formulation parameters when developing an ophthalmic drug delivery system (Sahoo et al., 2008)**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Criterias</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug</strong></td>
<td>Preferentially lipophilic. Non-ionisable lipophilic compounds will concentrate into the corneal epithelium, while ionisable lipophilic ones will partitionate into the aqueous humor.</td>
</tr>
<tr>
<td><strong>Vector Type</strong></td>
<td>Depends on encapsulated molecule. Should allow a high loading dose to reduce the instilled volume</td>
</tr>
<tr>
<td><strong>Carrier Size</strong></td>
<td>Lowest as possible to facilitate corneal uptake and passage</td>
</tr>
<tr>
<td><strong>Osmotic Pressure</strong></td>
<td>Isotonic with physiological fluids to avoid irritation and lacrimation</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>Close to physiological pH to avoid irritation and lacrimation. If buffering is necessary, the lowest possible buffer concentration is to be used (&lt;0.1 M)</td>
</tr>
</tbody>
</table>

Much of the published data suggests that in the case of ophthalmic drug delivery, an appropriate particle size and a narrow size range, ensuring low irritation, adequate bioavailability and compatibility with ocular tissues, should be sought for every suspended drug (Guinedi et al., 2005).

The use of nanotechnology-based drug delivery systems like nanosuspensions, solid lipid nanoparticles and liposomes can fulfil these needs. In addition to these points, encapsulation of drugs in nanospheres, liposomes, and so on, can also provide protection for the drug and hence prolong exposure of the drug by controlled release. Besides this, depending on their particle charge, surface properties and relative hydrophobicity, nanoparticles can be designed to be successfully used in overcoming retinal barriers (Kayser et al., 2005). These carriers are also very efficient in crossing...
membrane barriers, such as the blood retinal barrier in the eye (Sultana et al. 2006; Vandervoort et al., 2007).

The drug delivery systems based on nanotechnology may prove to be the best drug delivery tools for some chronic ocular diseases, in which frequent drug administration is necessary, for example in ophthalmic diseases like chronic cytomegalovirus retinitis (CMV).

Nanotechnology-based drug delivery is also suitable in the case of the retina, as it has no lymph system, hence retinal neovascularisation and choroidal neovascularization have similar environments to that of solid tumors, in which the EPR effect may be available for drug targeting by nanoparticles (Yasukawa et al., 2004).

Nanoparticulate technology is advocated as an ophthalmic drug delivery approach that may enhance dosage form acceptability while providing sustained release in the ocular milieu. Particle size, particle size distribution, and stability constitute a major issue considered by formulation scientists when formulating dispersed systems, especially those intended for parenteral or ocular administration. Very small particles such as nanoparticles are well tolerated and possess adhesive properties, which could prolong the residence time of the drug in the cul-de-sac, prevent tear washout (due to tear dynamics), and increase ocular bioavailability. Other potential advantages of nanoscaled drug delivery systems in ocular therapy are (1) the possibility of self-administration by patients as eyedrops; (2) no impairment of sight because of small dimensions of the delivery systems; (3) protection against metabolic enzymes (such as peptidases and nucleases); (4) possible uptake into corneal cells; (5) prolonged drug release, reducing the need for repeated instillation or injection; and (6) targeting toward affected tissues, reducing possible side effects and required dose (Araújo et al., 2009).

1.6 DIFFERENT NANOPARTICULATE BASED DRUG DELIVERY CARRIERS

The use of nanoparticulate drug delivery systems are 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
Various nanoparticulate-based drug delivery systems, like nanoparticles, nanoemulsions, nanosuspensions, liposomes, dendrimers, niosomes, Cyclodextrins and so on are used frequently for ophthalmic drug delivery.

### 1.6.1 Nanoparticles

Nanoparticles (NPs), as the very name implies, are particles varying in size from 10 to 1000 nm and depending on the end use, may or may not contain a drug molecule. The drug may be attached to a nanoparticle matrix, or dissolved, encapsulated and entrapped, giving rise to different terminologies as nanoparticles, nanospheres or nanocapsules (Fig. 2.6).

![Diagram of interactions of DNPs to ocular surface and subconjunctival space of human eye](image)

**Fig 1.6 Interactions of DNPs to ocular surface and subconjunctival space of human eye. (Nagarwal et al., 2009)**

For ophthalmic applications, properly formulated DNPs are reported to provide ease of application just like eye drop solutions, with the added advantage of being patient friendly, due to less frequent application and extended duration of retention in the extraocular portion.
Although the size of the nanoparticles are in the colloidal range which is more precisely accepted to fall between 1 nm and 0.5 μm for ophthalmic formulations, such a preparation may contain larger particles albeit within the colloidal range stated earlier.

### 1.6.1.1 Chitosan

Among the various polymeric materials available for formation of nanoparticles, 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 favourable biological properties (Lee et al., 1995).

Chitosan is obtained from the deacetylation of chitin, a naturally occurring and abundantly available (in marine crustaceans) biocompatible polysaccharide. However, applications of chitin are limited compared to chitosan because chitin is structurally similar to cellulose, but chemically inert. Acetamide group of chitin can be converted into amino group to give chitosan, which is carried out by treating chitin with concentrated alkali solution. Chitin and chitosan represent long-chain polymers having molecular mass up to several million Daltons. Chitosan is relatively reactive and can be produced in various forms such as powder, paste, film, fiber, etc. (Muzzarelli et al., 1986; Sjak-Braek et al., 1992). Commercially available chitosan has an average molecular weight ranging between 3800 and 20,000 Daltons and is 66% to 95% deacetylated.

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 immunostimulating activity. Chitin and chitosan have very low toxicity; LD50 of in laboratory mice is 16 g/kg body weight, which is close to sugar or salt. Chitosan is proven to be safe in rats up 10% in the diet (Arai et al., 1968). Various sterilization methods such as ionizing radiation, heat, steam and chemical methods can...
be suitably adopted for sterilization of chitosan in clinical applications (Chandy et al., 1990).

1.6.1.1.1 Derivatives of Chitosan

Chitosan, being a cationic polysaccharide in neutral or basic pH conditions, contains free amino groups and hence is insoluble in water. In acidic pH, amino groups of chitosan can undergo protonation thus, making it soluble in water. Solubility of chitosan depends upon the distribution of free amino and N-acetyl groups (Sannan et al., 1976). Usually 1–3% aqueous acetic acid solutions are used to solubilize chitosan.

1.6.1.1.2 Chitosan Formulations

1.6.1.2 Micro/nanoparticles prepared by ionotropic gelation method

Chitosan nanoparticles prepared by ionotropic gelation technique was first reported by Calvo et al., 1997 and has been widely studied (Janes et al., 2001; De la Funete et al., 2008) since then (Fig. 2.7). The mechanism of chitosan nanoparticles formation is based on electrostatic interaction between amine group of chitosan and negative charge group of a polyanion such as tripolyphosphate (Bodmeier et al., 1989; Calvo et al., 1997). This is a simple process with mild preparation condition. First, chitosan can be dissolved in acetic acid (or water, if it is a water soluble salt) in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers are then added. Nanoparticles are formed upon mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of and stabilizer (Calvo et al., 1997).

This method is one of the easiest methods amongst the various methods that are used for nanoparticles preparation viz. emulsion cross linking, coecervation/precipitation, spray-drying, reverse micellar method etc. Moreover, it is a safe method as it avoids the use of hazardous organic solvents while fabricating particles.
Chitosan/ Hyaluronic Acid Nanoparticles
As reported previously, chitosan is a linear polyamine containing a number of free amine groups that are readily available for cross-linking with multivalent anions. A particularly interesting formulation for the ocular delivery in this regard is developed by De la Fuente et al., which consists of a hybrid nanostructure of chitosan and the natural occurring glycosaminoglycan hyaluronic acid (HA) (De la Fuente et al., 2008).

Chitosan–lipid complexes
Another interesting carrier of chitosan with new and improved properties is developed by Sonvico et al., 2006. This nanosystem combines the positive features of polysaccharides with those of lipids. The nanoparticles obtained can be considered as a self-organized structure, result of the electrostatic interaction of the polycation chitosan and lecithin, due to the presence of negatively charged components in the lipid mixture (Sonvico et al., 2006).

1.6.2 Emulsomes
Emulsomes are solid fat nanoemulsions, represent a novel lipoidal vehicle of particulate structure with improved loading capacity for drugs and biologics containing components that have been safely used to deliver medications to people (Lowell et al., 1997).
1.7 APPLICATION OF NANOPARTICULATE CARRIER SYSTEMS FOR OCULAR DRUG DELIVERY

A nanoparticulate carrier system may be applied in liquid form like eye drop solutions, whereupon their interaction with the glycoprotein of the cornea and conjunctiva can form a precorneal depot resulting in prolonged release of the bound drug. Nanotechnology-based drug delivery is also very efficient in crossing membrane barriers, such as the blood-retinal barrier in the eye (Pignatello et al., 2002; Bucolo et al., 2004).

Gelatin nanoparticles encapsulating pilocarpine HCl (50%) or hydrocortisone (35% to 45%) as model drugs produced a sustained release for both drugs. In case of pilocarpine HCl-loaded spheres, no influence of the gelatin type or the pH level was observed, which could be attributed to the shielding effect of ions present in the dispersion medium. (Vandervoort and Ludwig, 2004).

Tissue concentration of progesterone following topical administration of nanoparticles was generally four to five times less than that observed with control solutions, due to the high affinity of progesterone for the nanoparticles, as the drug becomes less available for absorption during its residence time in the precorneal area.

Papadimitriou et al. 2006, prepared chitosan nanoparticles of dorzolamide hydrochloride (DZ) by ionic gelation method. The particle size of chitosan nanoparticles was affected by the CS/drug ratio but not by the type of drug. By increasing the ratio of CS/TPP, nanoparticles with smaller sizes are produced. DZ release followed a biphasic pattern, i.e. an initial rapid release followed by a period of slower release.

De la Fuente et al., 2008; De la Fuente et al., 2008b investigated the mechanism of action of a new type of nanoparticle made of two bioadhesive polysaccharides, hyaluronic acid (HA) and chitosan (CS), intended for the delivery of genes to the cornea and conjunctiva. The transfection studies showed that HA-CS nanoparticles provided high transfection levels (up to 15% of cells transfected), without affecting cell viability. They concluded that these nanoparticles may represent a new strategy toward the gene therapy of several ocular diseases.

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Diebold et al., 2007 evaluated liposomal-chitosan nanoparticles (LCS-NP) for delivery of drugs to the ocular surface. The conjunctival epithelial cell line IOBA-NHC was exposed to several concentrations of three different LCS-NP complex to determine the cytotoxicity.

### 1.8 DRUG PROFILE

#### 1.8.1 Ganciclovir

*(Clarke’s Analysis, 2006; Martindale, 2009; Merck Index, 2006; Drug bank, Rx Drug List, FDA Label)*

- **Category:** Antiviral
- **Synonyms:** BIOLF-62; BW-759; BW-B759U; BW-759U; DHPG; Dihydroxy-propoxymethylguanine; 2-NDG; 2-Nor-2-deoxyguanosine; RS-21592.
- **CAS Number:** 82410-32-0
- **Proprietary names across the globe:** Citovirax; Cymevan; Cymeven; Cymevene; Cytovene; Denosine; Virgan; Vitrasert.
- **Molecular formula:** C_{9}H_{13}N_{5}O_{4}
- **Molecular Weight:** 255.2

- **Structural Formula and Chemical Name:**
  2-Amino-1,9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-6H-purin-6-one

![Chemical structure of Ganciclovir](image)

- **Physicochemical Properties**
  - **Appearance and colour:** A white to off-white crystalline powder.
Solubility: Ganciclovir is a polar hydrophilic compound with 2.6 g/L solubility in water at 25°; 4.3 g/L in solution at pH 7.

Partition Coefficient: Log P (octanol/water), -1.66

Melting point: 250° (decomposes)

➢ IR Spectra of Ganciclovir:

![IR Spectra of Ganciclovir](image)

The empirical formula of ganciclovir is C₉H₁₃N₅O₄, structured as 2-Amino-1,9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-6H-purin-6-one. In FT-IR spectrm, the characteristic bands observed from data of ganciclovir include NH stretching in range of 3500-3300 cm⁻¹, CH stretching in range of 2960-2850 cm⁻¹, C=O stretching in range of 1760-1670 cm⁻¹, C=C stretching at 1606 cm⁻¹, C-O stretching in range of 1260-1000 cm⁻¹.

➢ Mechanism of action
Ganciclovir is an acyclic nucleoside analogue of 2'-deoxyguanosine that inhibits replication of herpes viruses. Ganciclovir has been shown to be active against cytomegalovirus (CMV) and herpes simplex virus (HSV) in human clinical studies.

To achieve anti-CMV activity, ganciclovir is phosphorylated first to the monophosphate form by a CMV-encoded (UL97 gene) protein kinase homologue, then to the di- and triphosphate forms by cellular kinases. Ganciclovir triphosphate concentrations may be 100-fold greater in CMV-infected than in uninfected cells,

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indicating preferential phosphorylation in infected cells. Ganciclovir triphosphate, once formed, persists for days in the CMV-infected cell. Ganciclovir triphosphate is believed to inhibit viral DNA synthesis by (1) competitive inhibition of viral DNA polymerases; and (2) incorporation into viral DNA, resulting in eventual termination of viral DNA elongation.

- **Disposition in the Body**
  - **After Oral Administration:**
    Ganciclovir is poorly absorbed after oral administration; peak plasma concentrations are reached after about 2 to 4 h. Following intravenous administration, it is widely distributed to body tissues and fluids including intra-ocular fluid and CSF. It crosses the placenta. The drug is excreted unchanged in the urine by glomerular filtration and active tubular secretion; approx. 90% of an oral dose is excreted in urine within 24 h of administration. With an intravenous dose, on the other hand, the majority is excreted in faeces. Haemodialysis reduces plasma concentrations by about 50%. Ganciclovir does not accumulate in plasma after repeated dosing.

- **After Intravenous Administration**
  - **Absorption**
    At the end of a 1-hour intravenous infusion of 5 mg/kg ganciclovir, total AUC ranged between 22.1 ± 3.2 (n=16) and 26.8 ± 6.1 μg·hr/mL (n=16) and Cmax ranged between 8.27 ± 1.02 (n=16) and 9.0 ± 1.4 μg/mL (n=16).

- **Distribution**
  The steady-state volume of distribution of ganciclovir after intravenous administration was 0.74 ± 0.15 L/kg (n=98). Cerebrospinal fluid concentrations obtained 0.25 to 5.67 hours postdose in 3 patients who received 2.5 mg/kg ganciclovir intravenously q8h or q12h ranged from 0.31 to 0.68 μg/mL representing 24% to 70% of the respective plasma concentrations. Binding to plasma proteins was 1% to 2% over ganciclovir concentrations of 0.5 and 51 μg/mL.

- **Elimination**
  When administered intravenously, ganciclovir exhibits linear pharmacokinetics over the range of 1.6 to 5.0 mg/kg and when administered orally, it exhibits linear kinetics.
up to a total daily dose of 4 g/day. Renal excretion of unchanged drug by glomerular filtration and active tubular secretion is the major route of elimination of ganciclovir. In patients with normal renal function, $91.3 \pm 5.0\%$ (n=4) of intravenously administered ganciclovir was recovered unmetabolized in the urine. Systemic clearance of intravenously administered ganciclovir was $3.52 \pm 0.80$ mL/min/kg (n=98) while renal clearance was $3.20 \pm 0.80$ mL/min/kg (n=47), accounting for $91 \pm 11\%$ of the systemic clearance (n=47). Half-life was $3.5 \pm 0.9$ hours (n=98) following IV administration and $4.8 \pm 0.9$ hours (n=39) following oral administration.

- **Toxicity:** The trough serum toxic concentration is 3 to 5 mg/L and peak, 20 mg/L.
- **Bioavailability:** 5 to 8%; increases when administered with food.
- **Half-Life:** Plasma half-life, 2.5 to 4 h.
- **Volume of Distribution:** 0.5 to 2.0 L/kg; quoted as 0.74 L/kg. Also reported as 33 to 45 L.
- **Clearance:** Plasma clearance, 200 mL/min/1.73 m$^2$; 0.1 L/h/kg.
  - **Protein Binding:** In plasma, 1 to 2%.

- **Analytical Methods:**
  A) **UV-Method**- A UV method was reported in Clarke’s Analysis for determination of ganciclovir using aqueous acid ($\lambda_{max} = 255$ nm); methanol ($\lambda_{max}$=254 nm) and aqueous base ($\lambda_{max}$=264nm) as solvents. The Ultraviolet Spectrum from clarke’s analysis is given below:
B) HPLC Methods

1) A simple HPLC method was reported by Shen et al., 2007 for determination of ganciclovir (Shen et al., 2007). In this work, the HPLC system consisted of a Waters 515 HPLC pump, Waters 2487 HPLC detector (Waters Corp, Milford, MA) set at 254 nm, and a Sanrui Chromatography Workstation (Sanrui Sci-Tech Co, Shanghai, China). The samples were chromatographed on a reversed-phase LiChrospher® C 18 column (150×4.6 mm, 5 μm, Jiangsu Hanbang SciTech Co, Huaiyin, China), and a 4×10mm precolumn of the same material. The mobile phase, at 1.0 mL/min flow rate, consisted of acetonitrile/water (0.4:99.6, vol/vol), which was filtered and degassed before use. The column was thermostated at 30°C, and under these experimental conditions, the run time was 18 minutes.

2) Another HPLC method for the assay of Ganciclovir was reported by Campanero et al., 1998. The method employed a RP Hypersil ODS (100 × 4.6 mm, 3 μm) column and a (pre-column) (10 × 4.6 mm). Column temperature: was maintained at 40°C. Mobile phase used was 0.1 M sodium hydrogen phosphate monohydrate: 0.04 M triethylamine, pH 6.6. Flow rate was 1.0 mL/min. Internal standard used was acyclovir. UV detection was made at 254 nm. Reported retention time of ganciclovir and interanal standard i.e., acyclovir was 4.0 min and 5.1 mins, resp.
1.8.2 Triamcinolone acetonide
(Martindale, 2009; Merck Index, 2006; Drug bank, Rx Drug List, FDA Label)

- **Category:** Corticosteroid, Anti-inflammatory Agent, Adrenergic Agent
- **Description:** A glucocorticoid given, as the free alcohol or in esterified form, orally, intramuscularly, by local injection, by inhalation, or applied topically in the management of various disorders in which corticosteroids are indicated. (From Martindale, The Extra Pharmacopoeia, 30th ed, p739)
- **CAS Number:** 76-25-5
- **Proprietary names across the globe:** Adcortyl; Aftab; Aftach; Albicort; Aristocort; Arutrin; Azmacort; Berlicort; Delphi(cort); Denkacort; Dermacort; Extracort; Facort; Generlog; Kanolone; Kela; Kemzid; Kenacort; Kenalog; Kenalone; Ledercort; Nasacor(t); Omcilon; Oracort; Orcilone; Sta-Cort A; Steronase; Triaderm; Triam; Triamonic; Tri-anemul; Triderm; Trigon; Trilog; Trimacort; Volon A; Volonimat; Zamacort.
- **Molecular formula:** \( C_{24}H_{31}FO_6 \)
- **Molecular Weight:** 434.50
- **Structural Formula and Chemical Name:**

![Chemical structure of Triamcinolone acetonide](image)

9-fluoro-11b,16a,17,21-tetrahydroxypregna-1, 4-diene-3,20-dione cyclic 16,17-acetal with acetone.

- **Physicochemical Properties:**
  - **Appearance and colour:** A white or cream-coloured crystalline powder.
Solubility: Very slightly soluble in water; soluble 1 in 150 of ethanol, 1 in 11 of acetone and 1 in 40 of chloroform; sparingly soluble in dehydrated alcohol and methanol.

Partition Coefficient: Log P (octanol/water), 1.2

Melting point: 292° to 294°.

IR Spectra of Triamcinolone acetonide

![Fig 1.12 IR Spectra of Triamcinolone acetonide](image)

The empirical formula of TA is C24H31FO6, structured as 9-fluoro-11,16,17,21-tetrahydroxypregna-1,4-diene-3,20-dione 16,17-cyclic acetal with acetone. In FT-IR spectrum (Fig.), the characteristic bands observed from the data of TA included the OH group in the range 3650–3200 cm⁻¹, C–H stretching of sp3 and sp2 carbons in the range of 3000 cm⁻¹ and 2900 cm⁻¹, C O in 1775–1650 cm⁻¹, C C in 1690–1635 cm⁻¹, C–O–C in 1310–1000 cm⁻¹ and a strong peak at 1050 cm⁻¹ due to stretching vibration of C–F.

Mechanism of Action:

Triamcinolone acetonide interacts with steroid cytoplasmic receptors to induce antiinflammatory effects; possesses antipruritic and antiinflammatory actions; The anti-inflammatory mechanism of corticosteroids remains to be fully elucidated. One that has been proposed is through the induction of lipocortin synthesis. Prostaglandins and leukotrienes, mediators of inflammation, have a common
precursor, arachadonic acid. Arachadonic acid is released from membrane phospholipids by phospholipase A2.3,24 Phospholipase A2 activity is inhibited by lipocortins.

In addition to their anti-inflammatory mechanism, corticosteroids exhibit vasoconstrictive effects that may contribute to their effectiveness in treating ocular neovascularization and lid hemangiomas (Sommer et al., 1998).

➢ **Disposition in the Body:**
Pharmacokinetic studies have been performed using a single oral dose of radiolabeled triamcinolone acetonide 800 mg given to healthy male volunteers. Relatively rapid absorption occurred after oral dosing with maximum plasma levels at 1.5 to 2 hours. Triamcinolone acetonide plasma protein binding is approximately 68%, relatively consistent over a wide plasma concentration range as a function of time. After oral dosing, metabolism and excretion are rapid and extensive with no detection of the parent compound in the plasma 24 hours post dose. Within 5 days greater than 90% of the oral dose was recovered in five of the six subjects in the study; 40% of the recovered radioactive marker was in the urine and 60% was in feces (Degenering et al., 2004).

Metabolism of triamcinolone acetonide yields three products. These factors are 6b-hydroxytriamcinolone acetonide, 21-carboxytriamcinolone acetonide, and 21-carboxy-6b-hydroxytriamcinolone acetonide. The metabolites are less active than the parent compound because: 1) anti-inflammatory activity depends on an intact 21-hydroxyl group, 2) 6-hydroxylation decreases activity, and 3) their increased water solubility markedly enhances their rates of elimination.3 It is important to note that these pharmacokinetic studies are based on oral administration.

In ophthalmic practice triamcinolone acetonide is used in its injectable form. The most commonly used dosages include 4 mg and 20 mg. Thus there may be fundamental differences in pharmacokinetics. One advantage to the higher dosage may be a longer duration of action (Jonas et al., 2004).

➢ **Toxicity:** $LD_{50} => 500\,\text{mg/kg}$ (in rats)

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- **Half-Life**: 88 minutes (oral)
- **Protein Binding**: 68% (oral)
- **Analytical Methods**:

A) **UV-Method** - A UV method was reported in Clarke’s Analysis for determination of triamcinolone acetonide using methanol ($\lambda_{max}=238$ nm) as solvent. The Ultraviolet Spectrum of triamcinolone acetonide from clarke’s analysis is given in Fig. 2.13.

B) **HPLC Methods**:
1) A simple HPLC method was reported by Araujo et al., 2011 for determination of triamcinolone acetonide in plasma samples. In this method, RP-HPLC system consisted of a Waters 1525 pump (Waters, Milford, MA) with a UV–vis 2487 detector (Waters) set at 254 nm. A reverse-phase column (Hypersil® ODS 5 µm, 10 × 0.46) with a flow rate of 1.5 mL/min was used. The mobile phase consisted of acetonitrile: methanol: water (30:10:60).

2) Another HPLC method was reported for the assay of triamcinolone acetonide by Liu et al., 2008. In this work, the HPLC system consisted of Agilent G1310A pump (Agilent 1100 Series, USA), an Agilent G1314A Variable Wavelength Detector set at 240nm and a Hypersil C18 column (5µm, 250mm×4.6 mm). The mobile phase was
a mixture of methanol, water and ether with a ratio of 65:35:4 (v/v/v). The HPLC analysis was performed at 25 °C with a mobile phase flow rate of 1 ml/min.
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