Formulation and Characterization of Nanovesicular Systems for Ocular Drug Delivery

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By

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Dedication

I dedicate this thesis to

my dear father, mother, husband,

brothers, sisters and daughters

ABSTRACT

The use of conventional eye drops for drug delivery usually results in poor ocular drug bioavailability. This is because of the natural protection mechanisms built in the eye which are responsible for the rapid elimination of foreign objects as well as drugs from the eye.

An approach utilizing methazolamide (MZA) loaded spanlastics vesicular systems (SVs) were prepared and tested for their efficiency. These systems consisted of Span 60 mixed with different ratios of edge activators (EA) namely: Tween 60, Tween 80, Brij 35 and Brij 58, dispersed in mucoadhesive *in-situ* gelling mixture composed of gellan gum and HPMC.

The tested systems were designed to combine high corneal penetration properties due to drug entrapment in highly deformable spanlastics vesicles, as well as ease of application and prolonged eye retention being instilled as eye drops that undergo sol-gel transition after being in contact with tear fluids.

MZA loaded SVs were successfully prepared by ethanol injection method and characterized for their entrapment efficiency percentages (EE %), particle size (PS), zeta potential (ZP), measurement of elasticity, *in vitro* release and physical stability studies.

Factorial design was used to study the influence of variables on PS and EE % namely; EA type with four levels Tween 60, Tween 80, Brij 35 and Brij 58 and Span 60: EA ratio with three levels 90:10, 80:20 and 70:30 (w:w). The results suggested the selection of the best suitable formulae for further investigations being with suitable EE % and PS. The formulae showed elastic properties, sustained release for 8 hrs and good physical stability.

MZA-SVs mucoadhesive *in-situ* forming gels were prepared by dispersion of selected MZA loaded SVs in mixture of gellan gum and HPMC. Results of viscosity showed that the best HPMC concentration was 0.5 w/v %, the release rates of MZA–SVs mucoadhesive *in-situ* forming gel formulae were found to be more sustained when compared to MZA–SVs formulae, all the prepared formulae had significant mucoadhesive properties after addition of HPMC.

Sterilization was carried out by gamma radiation. The EE%, PS and gelation time were measured after sterilization. The results showed non significant change in the EE% and the gelation time after sterilization by one of the following doses: 5, 15 and 25 KGy. There was no significant change in PS after using 5 KGy, however, there was a significant change in PS after exposure to 15 and 25 KGy of gamma irradiation used.

In vivo intraocular pressure (IOP) lowering activity for the selected formulae was performed on albino rabbits and compared to that of MZA solution. All the tested MZA-SVs mucoadhesive *in-situ* forming gel formulae showed significantly lowering in IOP values. The selected formula composed of SP 60: TW 60 (90:10), 0.6 w/v % gellan gum and 0.5 w/v % HPMC lowered the intraocular pressure by - 8.2 mmHg within 3 hrs and effect lasted for 12 hrs.

Keywords: Spanlastics; methazolamide; gellan gum; mucoadhesive, *in situ* gel; factorial design; intraocular pressure.

GENERAL INTRODUCTION

The eye ball is complex organ protected from exogenous substance and external stress by various anatomical and physiological barriers. The structure of the eye can be divided into two main parts: anterior segment and posterior segment.

Anterior segment of the eye occupies approximately one-third of eye volume while the remaining portion is occupied by the posterior segment.

Tissues such as cornea, conjunctiva, aqueous humor, iris, ciliary body and lens make up the anterior portion, however the posterior segment of the eye include sclera, choroid, retinal pigment epithelium, neural retina, optic nerve and vitreous humor (**Figure 1**).

Many diseases can affect the anterior segment of the eye including glaucoma, allergic conjunctivitis, anterior uveitis and cataract. However other diseases can affect the posterior segment of the eye including, age-related macular degeneration (AMD) and diabetic retinopathy ***(Le Bourlais et al., 1998)***.

Clinically, the eye was treated topically with commonly used eye drops, which are comfortable to patients ***(Urtti, 2006)***. Unfortunately various anatomical and physiological barriers restrict absorption of drug, causing drug loss, short duration of action, and poor drug bioavailability (< 5%). To increase the drug bioavailability and duration of action, the frequency of administered dose should be increased, which usually result in the decrease of patient compliance ***(Järvinen et al., 1995)***.

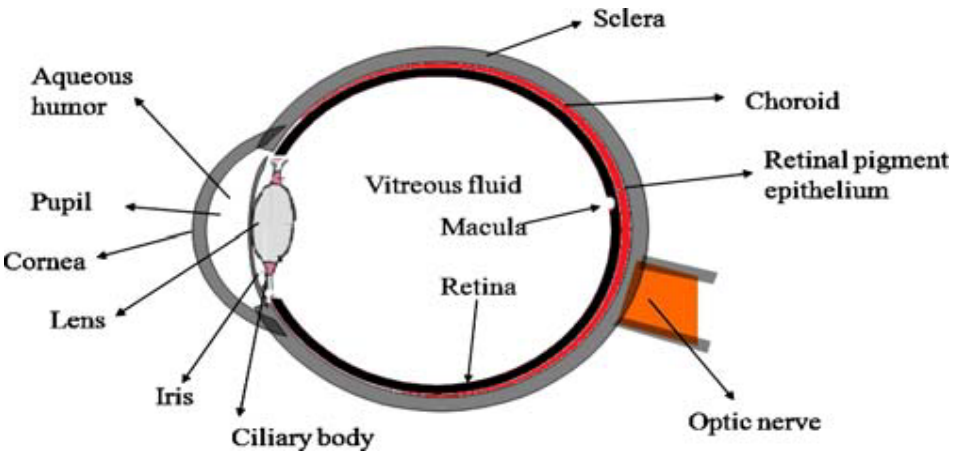

Figure 1: The structure of the eye (Gaudana et al., 2009).

Barriers which restrict intraocular drug transport

I- Tear

One of the precorneal barriers is tear film which consists of a superficial lipid layer, a central aqueous layer and an inner mucus layer. The *superficial* lipid layer is secreted during blinking by the meibomian glands embedded in the tarsal plate of the eyelids, and the accessory sebaceous glands of Zeiss.

It spreads over the aqueous layer during eye opening. It consists mainly of sterol esters, triacylglycerols, phospholipids, free sterols and free fatty acids. It plays an important role in reducing the evaporation rate and maintaining the normal tear osmolality even when the tear flow is quite low.

The *second* layer is aqueous layer. It is secreted from the lachrymal gland and accessory gland. The composition of this layer usually includes inorganic salts, glucose, urea, retinol, ascorbic

acid, various proteins, immunoglobulins, lysozyme, lactoferrin and glycoproteins.

The mean pH value of normal tears is about 7.4, depending on age and diseases, however, values between 5.2 and 9.3 have been also measured ***(Baeyens and Gurny, 1997)***.

The *third* layer is mucus layer which is secreted by goblet cells. The primary component of this layer is mucin.

Mucin is a high molecular mass glycoprotein with subunits containing a protein core, approximately 800 amino acids long, of which about 200 are bearing polysaccharide side-chains. Mucin carries negative charge at physiological pH.

The mucus layer is very sensitive to hydration and forms a gel layer with viscoelastic rheological properties. It protects the outer epithelial layer of cornea from damage and facilitates the movements of the eyelids.

Mucin also improves the spreading of tear film and enhance its stability and cohesion. It also acts as a defense against pathogens.

Tear film affect drug concentration by dilution, clearance due to tear turnover (~1.2µl /min), or by binding of drug molecules to tear proteins. Also the volume of administered dose ~50µl, is much larger than the size of cul-de sac which is only 7-10 µl, thus the excess volume may pass from nasolacrimal duct or spill out ***(Greaves and Wilson, 1993; King-Smith et al., 2000)***. Also the blinking action of the eye affect ocular drug concentration and the rheological properties of the viscous ocular dosage forms instilled and consequently the drug bioavailability ***(Ludwig, 2005; (Balasubramaniam et al., 2003)***.

II- Cornea

The cornea consists of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium **(Figure 2)**.

Among these layers epithelium, stroma, and endothelium are important in our study, each layer has different polarity and rate limiting for drug permeation, the epithelium is lipophilic in nature and have tight junction that restricts paracellular drug penetration ***(Kaur and Smitha, 2002)***.

The tight junction complex includes two integral transmembrane proteins (claudin and occludin) and the membrane-associated protein ZO-1 ***(Ban et al., 2003)***.

The low permeability of the cornea is due to the presence of tight junctions between the cells, which act as a selective barrier for small molecules and prevents the diffusion of macromolecules via the paracellular route.

The stroma is an extracellular matrix of lamellar arrangement of collagen fibrils, highly hydrophilic in nature hence restricts the permeation of lipophilic drugs. The endothelium is lipophilic in nature which act as separating barrier between the stroma, and aqueous humor ***(Almeida et al., 2014).***

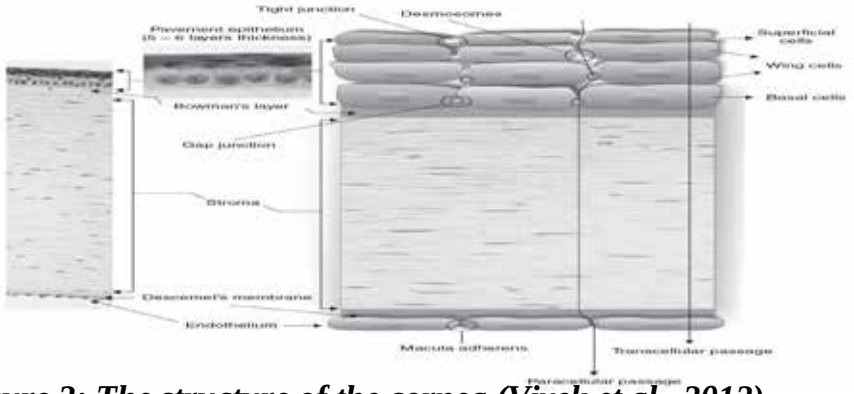

Figure 2: The structure of the cornea (Vivek et al., 2012).

III- Conjunctiva

It is a thin transparent membrane, responsible for the formation and maintenance of tear film. The goblet cells are an important anatomical element of conjunctiva.

The conjunctiva has a rich supply of capillaries and lymphatics, thus it represent a pathway through which drug may be cleared to systemic circulation. The conjunctival blood vessels don't form tight junction barrier, hence drug can enter to circulation by pinocytosis and/or convective transport through paracellular pores in the vascular endothelial layer ***(Singh et al., 2003)***. Conjunctiva is permeable to hydrophilic and large molecules, thus it plays an important role in the absorption of proteins and peptides. However, corneal permeation route is currently dominating because most drugs that is used clinically are small and fairly lipophilic ***(Kuno and Fujii, 2011).***

IV- Blood ocular barriers

The blood ocular barriers consist of two parts, blood aqueous barrier, and blood retinal barrier. The blood aqueous barrier prevents the access of plasma albumin into the aqueous humor and also limits the access of hydrophilic drugs from plasma to the aqueous humor. The blood retinal barrier is between the blood stream and eye and consists of Retinal Pigment Epithelium (RPE) and the tight walls of retinal capillaries. The two functional barriers restrict the movement of blood elements to the intraocular chambers and this explains why drugs administered orally or intravenously can hardly reach therapeutic levels in intraocular tissues ***(Occhiutto et al., 2012).***

Topical ocular drug delivery systems

The most common, acceptable, inexpensive, and comfortable route with ease of administration for drug delivery to patients is topical route. An ideal topical ophthalmic formulation would enhance bioavailability by facilitating corneal penetration and maintaining the contact of drug with the eye for prolonged periods of time ***(Le Bourlais et al., 1998)***.

A. Eye drops

Most ocular diseases are treated with topical application of solutions administered as eye drops. These conventional dosage forms account for nearly 90% of the currently accessible ophthalmic formulations. Eye drops have many advantages including low cost, safety, immediate actively, simplicity of formulation development and good acceptance by patients ***(Lang, 1995)***.

The major problems encountered with the topical eye drops is their rapid and extensive precorneal loss caused by drainage and high tear fluid turnover. After instillation of an eye drop to the ocular cavity, typically less than 5% of the applied drug penetrates the cornea and reaches intraocular tissues, however, a major fraction of the instilled dose is often absorbed systemically via the conjunctiva and nasolacrimal duct. It is well known that normal dropper used with conventional ophthalmic solution delivers about 50-75µl per drop and large portion of these drops quickly drain until the eye is back to normal resident volume of 7µl. Because of the large loss of drug in front of the eye, very little drug is available to enter the cornea and inner tissue of the eye. Another problem is a pulse entry of the drug, followed by rapid decline in drug concentration ***(Le Bourlais et al., 1998)***.

Therefore, to improve drug contact time, permeation and ocular bioavailability, various additives may be added to topical eye drops such as viscosity enhancers e.g. hydroxy methyl cellulose, hydroxy ethyl cellulose, sodium carboxy methyl cellulose and hydroxypropyl methyl cellulose which improve precorneal residence time. Permeation enhancers e.g. surface active agents can improve corneal uptake by reducing the thickness of mucus and breaking junctional complexes ***(Ludwig et al., 1990)***.

B. Eye ointments

Eye ointments are usually formulated using mixture of semisolid and solid hydrocarbons (paraffins) which have a melting or softening point close to the body temperature and are nonirritating to the eye.

Medicinal agents are added to ointment bases either as solution or as finely micronized powder. Eye ointments are useful as sustained drug release dosage form being broken down to small droplets after their instillation in the eye and hence act as drug reservoir in cul- de- sac and enhance drug bioavailability.

Although eye ointments are safe and well tolerated by the eye, they usually result in poor patient compliance as it may induce blurred vision and occasional irritation ***(Sasaki et al., 1999).***

C. Ocular inserts

Solid ocular dosage forms such as films, inserts, rods, and shields were developed to overcome the disadvantages associated with conventional ocular dosage forms like eye drops (aqueous solutions, and suspensions) and eye ointments.

Ocular inserts provide more controlled, sustained, and continuous drug delivery by maintaining effective drug concentration in the ocular tissues yet minimizing the frequency of applications.

Also inserts reduce the systemic absorption of many drugs, giving accurate drug dose in contrast to eye drops which provide pulse entry pattern of the drug after each administration to the eye which may cause transient overdose ***(Patel et al., 2010)***.

A number of ocular inserts using different techniques, namely soluble, erodible, non-erodible, and hydrogel inserts made from different polymers such as cellulose derivatives, acrylates, and poly (ethylene oxide), have been investigated over the last few decades.

However, ocular inserts have many disadvantages like patient incompliance, difficulty with self-insertion, foreign body sensation, and inadvertent loss from the eye ***(Sasaki et al., 1999; Patel et al., 2010)***.

D. Microemulsions

Microemulsions are dispersions of water and oils, with droplet size in dispersed phase <1.0μm in size. They are prepared using surfactant and co-surfactant agents as stabilizing agent.

Microemulsions are known to be transparent in appearance, thermodynamically stable, and inexpensive. In addition, they can be used in topical ocular drug delivery because of their stability, ease of preparation and sterilization, and their high capacity for dissolving drugs ***(Vandamme, 2002)***. Moreover, microemulsion formulations can be used as carriers for poorly water soluble drugs because of their structure which allows solubilizing lipophilic drugs in their oil phases ***(Gan et al., 2009)***.

The presence of surfactant and co-surfactant in the composition of microemulsion usually act as penetration enhancer, which increase membrane permeability thereby increasing drug uptake. Another advantage that microemulsions can achieve is the sustainment of drug release when applied to the cornea and the

higher penetration into the deeper layers of the ocular structure and the aqueous humor when compared to the native drug ***(Lawrence and Rees, 2000; Sahoo et al., 2008)***.

Pilocarpine-based micro- emulsions were found to delay the activity of the drug thus decrease frequency of its instillation in the eye. It was found that four times daily dosing regimen of conventional pilocarpine eye drops is equivalent to twice daily dosing regimen of microemulsion system ***(Naveh et al., 1994)***. Although microemulsion has many advantages, however, potential toxicity associated with higher concentrations of surfactant/co-surfactant in microemulsion composition often restricts its use.

E. Nanoparticles

Nanoparticles are sub micrometer sized polymeric colloidal particles used to improve drug bioavailability ranging in size from 10 to 1000nm (1µm).

They consist of macromolecular materials in which the drug is dissolved, entrapped, encapsulated, and/or to which the drug is adsorbed or attached ***(Le Bourlais et al., 1998)***.

They can be classified into two groups, nanospheres and nanocapsules.

Nanospheres are small solid spherical particles composed of dense solid polymeric network, developing over a large specific area ***(Rollot et al., 1986).*** Drugs can either be incorporated in the matrix of the nanospheres or adsorbed onto the surface of the colloidal carrier. Nanocapsules are small capsules formed of a central cavity surrounded by a polymeric membrane.

The major limiting issues for the development of nanoparticles include the control of particle size and drug release rate.

F. Vesicular systems

i. Liposomes

Liposomes are microscopic vesicles composed of membrane like lipid bilayers separated by water or aqueous buffer compartments. They are composed of lipids similar to those present in biological membranes with diameter ranging from 80 nm to 10 μm.

Liposomes first described by Bangham ***(Bangham et al., 1965)*** were able to entrap both hydrophilic and lipophilic drugs, depending on the nature of the loaded drug. Thus they can be used as carriers for water-insoluble drugs in liquid dosage form ***(Shell, 1985)***.

According to their size, liposomes are classified to either small unilamellar vesicles (SUV) (10-100nm) or large unilamellar vesicles (LUV) (100-3000nm). If more than one bilayer is present then they are called multilamellar liposomal vesicles (MLV) **Figure 3** ***(Kaur et al., 2004).***

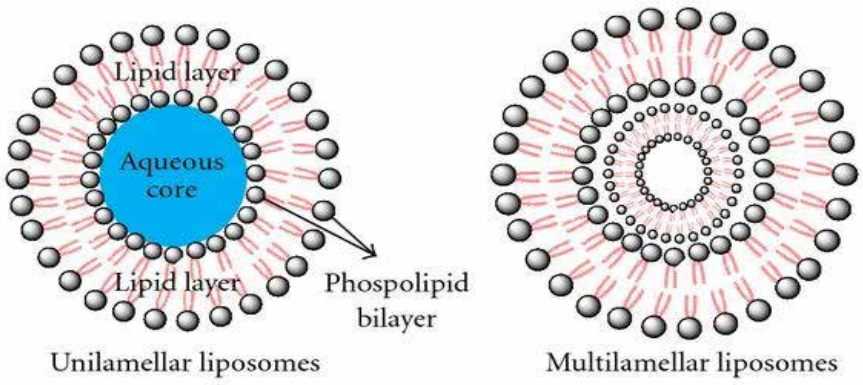

Figure 3: Schematic representation of unilamellar and multi-lamellar liposomes.

Liposomes have many advantages being completely biodegradable, relatively nontoxic and able to stabilize drug