REVIEW ARTICLE

Topical Ocular Delivery of Nanocarriers: A Feasible Choice for Glaucoma Management

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Abstract: Topical ocular delivery is an acceptable and familiar approach for the treatment of common ocular diseases. Novel strategies for the treatment of inherited eye diseases include new pharmacologic agents, gene therapy and genome editing, which lead to the expansion of new management options for eye disorders. The topical ocular delivery of nanocarriers is a technique, which has the potential to facilitate novel treatments. Nanocarrier-based strategies have proven effective for site-targeted delivery. This review summarizes recent development in the area of topical delivery of different nanocarriers (Polymer, Vesicular and dispersed systems) for the management of glaucoma, a group of ocular disorders characterized by progressive and accelerated degeneration of the axons of retinal ganglion cells, which make up the optic nerve. Unique cellular targets for glaucoma treatment, primarily the trabecular meshwork of the anterior segment of the eye, make glaucoma facilitated by the use of nanocarriers an ideal disorder for novel molecular therapies.

1. INTRODUCTION

Glaucoma is a group of neurodegenerative disorders of the eye that leads to damage of the optic nerve and vision loss. In 2020, over 80 million people throughout the world are affected by open-angle glaucoma and angle-closure glaucoma, while by the end of 2040 it is expected that more than 111 million individuals will have glaucoma [1]. The existing management of glaucoma is generally targeted at reducing the intraocular pressure (IOP), by using topical eye drops containing IOP reducing agents. Topical drop administration has limitations including poor corneal penetration, precorneal tear clearance, and a short preocular retention period, all of which reduce ocular bioavailability. Therefore, frequent administration is required, which results in poor patient compliance [2].

One of the most attractive areas of research in drug delivery is the design of nanosystems that are able to facilitate the delivery of drugs to the correct tissue and cells, at appropriate times and at the correct dose. Nanocarriers are submicron particles containing entrapped drugs intended for enteral or parenteral administration, which may prevent or minimize drug degradation and metabolism as well as cellular efflux [3]. Nanoparticles also have a long shelf-life, are made of safe materials, including synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides, and have the potential for overcoming important mucosal barriers, such as intestinal, nasal, and ocular barriers [4].

The topical application of drugs to the surface of the eye is the most common treatment modality for numerous ophthalmic disorders. The major obstacle encountered with conventional topical delivery of ophthalmic drugs is the rapid and extensive pre-corneal loss caused by drainage and high tear fluid turnover, resulting in only 5% of the applied drug penetrating the cornea/sclera and reaching the targeted intraocular tissues [5]. Accordingly, one of the major challenges facing pharmaceutical formulators is the development of topical ocular delivery systems with improved ocular retention, increased corneal/sclera drug absorption and reduced systemic side effects, while maintaining safety, simplicity and convenience. Many strategies have been adopted to partially or fully achieve such objectives [6-8]. However, the short residence time of these colloidal carrier systems in the ocular mucosa presents a major challenge for the therapy of most eye diseases. Consequently, the design of a mucoadhesive carrier system with improved drug delivery properties to the ocular surface would be a promising step towards the management of ocular diseases.

This manuscript reviews nanocarriers, which have proven helpful in the management of glaucoma *via* topical ocular delivery. We focus on different nanocarriers, which are categorized into polymeric nanocarriers, vesicular nanocarriers and dispersed systems (Fig. **1**). A segment of the manuscript has been devoted to positively charged nanocarriers for topical ocular delivery. Furthermore, we have incorporated the most recent related research.

2. ANATOMY AND PHYSIOLOGY OF THE EYE

The anterior segment of the eye consists of the cornea, iris, lens, and aqueous humor. The cornea is transparent and composed of five layers: the epithelium, Bowman's membranes, stroma, Descemet's membrane, and endothelium [9]. The epithelium layer of the cornea is a rate-limiting barrier for the transcorneal diffusion of most hydrophilic drugs [10]. The tight junctions found in the corneal epithelium serve as a selective barrier for small molecules and prevent the diffusion of macromolecules through the paracellular route [11]. The stroma represents 90% of the total corneal thickness and is composed of collagen fibrils arranged in a crystalline pattern, which hinders the transport of hydrophobic molecules [12].

The conjunctiva is a thin and transparent membrane lining the inner side of the eyelids which covers the anterior surface of the sclera, extending to the edge of the cornea (the limbus). The mucous membrane of the conjunctiva is composed of three layers: an outer epithelium (which acts as a permeability barrier), substantia

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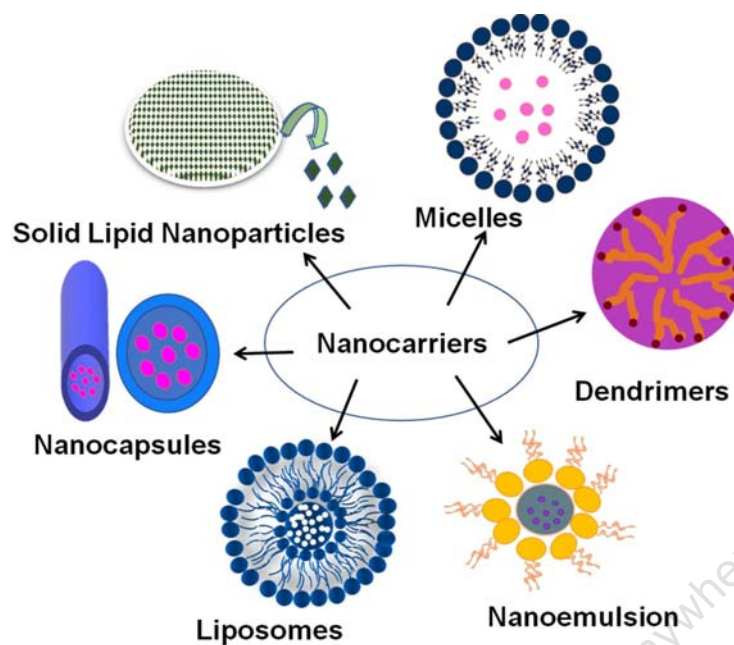
Fig. (1). Schematic representation of the different nanocarriers for ocular delivery. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

propria, (containing nerves, lymphatics and blood vessels) and the submucosa, which provides a loose attachment to the underlying sclera [13].

The sclera is structurally similar to the corneal stroma having numerous channels and consisting mainly of collagen and muco-polysaccharides. The sclera is in continuation of the cornea and extends posteriorly from the limbus. The sclera, which is poorly vascularized, is significantly more permeable than the cornea, but less permeable than the conjunctiva [14, 15]. The uvea is the vascular layer of the eye consisting of iris, ciliary body and choroid. The iris is the interior portion of the uvea and is immersed in the aqueous humor. It controls the amount of light rays that stimulate retina photoreceptors [16]. The choroid is a highly vascularized structure comprising the posterior portion of the uvea and functions in providing oxygen and nutrients to the outer third of the retina [17].

The crystalline lens is a transparent biconvex structure, which is located behind the iris and is composed of fibers from epithelial cells. It separates the aqueous humor from the vitreous humor. It is characterized by its plasticity and its ability to alter the curvature radius and refractive index, allowing active control of light penetration [18].

The ciliary body is formed by several biological regions, which include the non-pigmented ciliary epithelium, pigmented ciliary epithelium, stroma and ciliary muscle. It is fenestrated and leaky capillaries confer intercommunication of the anterior and posterior chambers [19], at which the aqueous humor is secreted into the posterior chamber and flows through the pupil into the anterior chamber. The vitreous humor is a clear aqueous gel in which the matrix-forming polymer system consists mainly of collagen and hyaluronic acid. The retina, the innermost eye tissue is highly a differentiated neuronal structure consisting of the retinal pigmented epithelium (an outer layer) and the neuro-epithelium (an inner layer), which consists of photoreceptors (rods and cones), horizontal cells, bipolar cells and retinal ganglion cells (among others), where the functions of light reception and transmission are located. The vitreous constitutes two-thirds of the whole eye and is a transparent, colorless and gelatinous mass, which is composed of 99% water [18].

3. FACTORS LIMITING BIOAVAILABILITY OF TOPICAL OCULAR DELIVERY

3.1. Tear Film

Tears are secreted from the lachrymal gland and have a vital role in maintaining normal eye function. Tears are mainly composed of water, electrolytes, lipids, proteins, glucose and mucins [20]. A thin film is formed by tears which hydrates and protects the ocular surface from desiccation [21] and bacterial infections [22]. While tears are an essential component of normal ocular function, they have a negative effect on ocular drug bioavailability. The conjunctival cul-de-sac can transitorily contain about 30 µl of tears, whereas the normal human tear volume is estimated to be 7 µl, and the typical volume administered by commercial eyedroppers is 35 - 56 µl, which leads to a sudden increase in the tear volume resulting in a rapid blinking reflex [23]. Drugs, which may be an irritant to the eye, cause increased secretion of tears resulting in an additional diluting effect and poor ocular bioavailability. Additionally, tears are characterized by a high turnover rate, which limits the ocular residence time for a drug to 5-6 minutes, consequently minimizing the period during which the drug can penetrate ocular tissues [24]. The presence of proteins and mucins in tears also negatively impacts drug bioavailability as they bind to drug molecules and reduce the effective concentration in contact with the cornea [25].

3.2. Corneal Barriers

Physiologically, the corneal epithelium is the most critical barrier to penetration of solutes into the eyes. The tight intercellular junctions (pore size) are formed between epithelial cells resulting in a tight diffusion barrier for the absorption of drugs from the tear fluid to the anterior segment of the eye. Drugs passing through the cornea depend on the physicochemical properties of molecules, and their molecular radius or molecular weight. As such, the corneal epithelium and endothelium allow the passage of small lipophilic molecules but restrict hydrophilic molecules from the aqueous humor [26]. In contrast to the cornea, the stroma resists the passage of lipophilic molecules and allows diffusion of hydrophilic substances. The conjunctiva consists of two layers, the outer epithelium, which acts as the major penetration barrier, and its underlying stroma. The

permeability of drugs across the conjunctiva is greater than across the cornea as the intercellular gaps in the conjunctival epithelium are much wider than the corneal epithelium [27].

3.3. Blood-ocular Barriers

Blood-ocular barriers include the blood-aqueous barrier (BAB) and the blood-retinal barrier (BRB). The BAB protects the intraocular environment and limits the passage of drugs from the anterior to the posterior segment. The endothelium of the iris-ciliary blood vessels and the non-pigmented ciliary epithelium layers form the BAB in the anterior segment of the eye. The BAB contributes nutrition to the cornea and the lens. The BAB is strongly impacted by the molecular weight of the solute, accordingly the solute concentration in the aqueous humor decreases with increasing molecular weight, signifying the presence of a selective barrier or molecular sieve [28]. Therefore, conventional topical dosage forms fail to provide the required drug concentrations for the treatment of posterior segment diseases [29].

The retina is a thin transparent tissue, which is considered a significant barrier to drug penetration [30]. The BRB is formed by the tight junctions between the endothelial cells of the retinal vessels (the inner BRB) and by similar tight junctions in the retinal pigment epithelium (the outer BRB) [31]. The BRB mainly restricts the diffusion of substances from the systemic circulation into the retina [32]. Small molecules such as glucose and ascorbate are permeable through the inner BRB, whereas the BRB is impermeable to larger molecules of 20-30kDa or greater. The outer BRB consists of retinal pigment epithelial cells with tight intercellular junctional complexes known as the zonula occludens. Active transport of molecules takes place across the BRB from the vitreous into the blood, and the passive transfer takes place into the vitreous, which is dependent on a concentration gradient [31].

4. TYPES OF GLAUCOMA AND TREATMENT APPROACH

Glaucoma has been widely categorized as acute and chronic, secondary and primary. Generally, glaucoma is classified into three major types: Primary Open Angle Glaucoma (POAG), Primary Angle Closure Glaucoma (PACG), and Primary Congenital Glaucoma (PCG) [33]. POAG is the most common type of glaucoma accounting for approximately 80% of all glaucomas [34]. In cases of POAG, the precise mechanism of neurodegeneration and IOP elevation are not fully known [35]. Elevated IOP is a common risk factor that connects nearly all forms of glaucoma.

4.1. Primary Open Angle Glaucoma (POAG)

POAG is the most common type of glaucoma in most populations. The "open" drainage angle of the eye becomes blocked, which leads to gradual IOP elevation. When this increased pressure damages the optic nerve, it is known as chronic open-angle glaucoma. Usually damage to the optic nerve and vision loss occur gradually and are painless [36]. There have been two major theories proposed for mechanisms of retinal ganglion cell death in POAG: (i) Mechanical and (ii) Vascular theories.

4.1.1. The Mechanical Theory

The mechanical theory hypothesizes that an elevated IOP reduces the structure in and around the optic nerve head, disturbing the axoplasmic transport within the nerve fibers. This causes the death of retinal ganglion cells (RGCs) and their axons, leading to the thinning of the neuroretinal rim and excavation of the optic nerve head ('cupping') [37].

4.1.2. The Vascular Theory

In the vascular theory, glaucomatous optic neuropathy is considered to be a result of an insufficient blood supply either due to increased IOP or other causes that reduce ocular blood flow (OBF) such as elevated systemic blood pressure or vasospasm. This causes

a relative reduction of the ocular perfusion pressure (OPP), which is the difference between systemic blood pressure and IOP. With this vascular theory, it has been hypothesized that both elevated IOP and reduced OBF are associated with the progression of glaucoma [38].

4.2. Primary Angle Closure Glaucoma (PACG)

Angle closure glaucoma, also known as closed-angle glaucoma, occurs when the iris swells forward to narrow or block the drainage angle formed by the cornea and iris. This hinders the circulation of fluid through the eye resulting in increased pressure. Angle-closure glaucoma may occur suddenly (acute angle-closure glaucoma) or slowly (chronic angle-closure glaucoma). Acute angle glaucoma is a medical emergency [39].

4.3. Treatment Approaches for Glaucoma

The medicinal treatment of glaucoma includes drugs of various classes such as prostaglandin analogs, beta-blockers, carbonic anhydrase inhibitors, adrenergic agonist, miotics and hyperosmotic agents. These classes of drugs either increase the flow of fluid from the eye or reduce the production of the fluid in the eye [40]. Topical beta-blockers have been the first-line drugs and include timolol, levobunolol, carteolol, metipranolol, and betaxolol. They lower IOP by reducing aqueous formation by downregulation of adenylyl cyclase in the ciliary body. Prostaglandin analogs including latanoprost, travoprost, tafluprost, unoprostone, and bimatoprost have recently become preferred drugs. They act by increasing uveoscleral outflow of aqueous humor by increasing the permeability of tissues in the ciliary muscle or by an action on episcleral vessels. α-adrenergic agonist agents, such as brimonidine and apraclonidine, are also used for glaucoma treatment. They reduce IOP by a dual mechanism of action; reducing aqueous humor production and increasing aqueous humor outflow *via* the uveoscleral pathway. The carbonic anhydrase inhibitor class of drugs (dorzolamide, brinzolamide and acetazolamide) acts by reducing aqueous humor production by limiting the generation of bicarbonate ion in the ciliary epithelium [41]. Since the 1970s, topical pilocarpine or anticholine esterase was the standard antiglaucoma drug. They lower IOP by increasing ciliary muscle tone increased aqueous humor outflow [42].

Surgical treatments of glaucoma include trabeculoplasty and iridotomy. Trabeculoplasty is a laser surgery method that enhances aqueous humor drainage to control IOP when treating open-angle glaucoma. Iridotomy is a laser treatment that creates minute holes in the iris to improve the flow of aqueous humor to treat narrow angle glaucoma. Surgery is recommended only if the ophthalmologist feels the benefit of a lower IOP achieved with an operation outweighs possible complications and/or further progression of optic nerve damage [43].

5. NANOCARRIERS BASED APPROACH FOR MANAGEMENT OF GLAUCOMA

The design of nanocarriers is an important area of research for ocular drug delivery with the goals of delivery of drugs to the correct site, at specific times, and exact dosages. The submicron size range of nanocarriers enclosed drugs intended for the oral or parenteral route of administration may specifically avoid or reduce drug degradation and metabolism, as well as control cellular efflux [44]. Nanocarrier-based ophthalmic delivery can overcome various shortcomings of conventional ocular drug delivery systems including insolubility in water, eye irritation, and drug efficacy helping to achieve low dose administration. The design of mucoadhesive nanocarriers can increase the contact time on the ocular surface, which may lead to controlled drug delivery for prolonged periods [45].

5.1. Polymer-based Nanocarrier Systems

5.1.1. Nanoparticles

Nanoparticle systems have been extensively studied for drug delivery to the eye. They are defined as polymeric colloidal nanoparticulate systems ranging in size from 10 to 1000 nm, in which the active substance may be either solubilized, dispersed, encapsulated or adsorbed [46]. Depending on the preparation methods, different types of nanometric systems can be obtained, such as nanospheres or nanocapsules.

Nanosphere-based systems consist of matrix type structures in which the active substance can be dissolved, dispersed in the matrix, or firmly adsorbed on to the surface of the matrix. In the case of nanocapsules polymer coat and core systems, the drug substance can be solubilized in the inner core or adsorbed onto the surface coat [47]. In order to attain a sustained drug delivery and extended therapeutic efficacy, nanoparticles require a bioadhesive on the conjunctival cul-de-sac, and the incorporated drug must be delivered from the nanocarrier at a specific rate. If the drug release rate is too high, there is no prolongation in the release of the drug. In cases where the drug release is slow, a beneficial effect of drug concentration miscible with tears can facilitate bioavailability to ocular tissues [48].

Chitosan-based nanoparticles have numerous beneficial biological properties such as bioadhesive, improved penetration power, nontoxicity, non-immunogenic, noncarcinogenic, antibacterial, and biocompatibility with ocular tissues and barriers. They have the ability to penetrate the corneal layer through a transcellular pathway process and a precise attraction for some conjunctival cells. Chitosan nanoparticles as vital systems for ocular drug delivery have been reported [49].

Siafaka *et al*. developed plain chitosan and its derivatives with succinic anhydride and 2-carboxybenzaldehyde nanoparticles. Nanoparticles were prepared *via* the ionic gelation method with sodium tripolyphosphate as a cross-linking agent. The formed nanoparticle size was directly dependent on the chitosan and their ratios with the cross-linking agent. Timolol maleate, which is appropriate for the management of glaucoma, was successfully encapsulated in these nanoparticles. The release of drugs from nanoparticles depends on particle sizes, nature of the drug, and the swelling degree. The combination property of mucoadhesion and swelling nature of chitosan derivative nanoparticles proved successful for the ocular bioavailability of timolol maleate [50].

Carteolol loaded chitosan nanoparticles were prepared by the ionotropic gelation method and their potential for ocular drug delivery was assessed. Nanoparticles showed a particle size of 243 nm, and drug loading and entrapment efficiency were $49.21 \pm 2.73\%$ and $69.57 \pm 3.54\%$, respectively. Drug release from nanoparticles was sustained for 24 h and showed good permeation with non-significant changes to the cornea. Carteolol loaded nanoparticles reduced IOP for long periods of time as compared to aqueous carteolol solution due to enhanced residence time on corneal and conjunctival surfaces, which was determined by γ-Scintigraphy [51].

Katiyar *et al*. investigated an *in situ* gel of chitosan nanoparticles to enhance the bioavailability and efficacy of dorzolamide in the treatment of glaucoma. Mucoadhesive testing and the HET-CAM assay showed the mucoadhesive and non-irritant nature of nanoparticles. *In vitro* release studies were performed to show the amount of drug released from the formulation with respect to time, while *ex vivo* permeation studies were performed to show the rate at which drug permeates across a unit area of biological membrane. The *ex vivo* release of the optimized *in situ* gel nanoparticle formulation showed sustained drug release as compared to the marketed formulation. The gamma scintigraphic study of prepared *in situ* nanoparticle gel showed good corneal retention compared to the marketed formulation [52].

Chitosan-sodium alginate nanoparticles loaded with timolol maleate were developed by the pre-gelation method, as a targeted drug delivery carrier for the treatment of glaucoma. The morphology and size of nanoparticles were spherical and in the range of 80-100 nm. The loading capacity and encapsulation efficiency were about 42% and 94%, respectively. The corneal penetration of timolol maleate loaded in nanoparticles was twice than that of timolol maleate solution [53].

Warsi *et al*., studied the effect of two different emulsifiers (PVA and vitamin E TPGS) on the development of dorzolamide loaded polylactic-co-glycolic acid nanoparticles by a modified double emulsion-solvent evaporation method. The effects of various formulation and process variables on particle size and encapsulation efficiency were assessed. Nanoparticles emulsified with vitamin E TPGS were found to possess enhanced drug encapsulation as compared to those developed with PVA as the emulsifier. The authors concluded that vitamin E TPGS is a safer and more effective emulsifier compared to traditional PVA. Being a potent emulsifier (77 times that of PVA), vitamin E TPGS provides better encapsulation of the drug and also acts as a P-glycoprotein inhibitor. P-glycoproteins are prominent efflux transporters identified in ocular tissues. Hence, a viable strategy to improve the ocular absorption of topically administered drugs that are substrates for efflux proteins would be to inhibit efflux pumps on the cornea [54].

5.1.2. Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are one of the most important drug delivery systems for the treatment of ocular diseases. The dispersed solid lipid core assemblies are stabilized by surfactant in an aqueous medium, and are capable of accommodating lipophilic or hydrophilic drug moieties. SLNs have various advantages including safeguarding the loaded drug, preparation without organic solvents and, easy manufacturing with appropriate methods. SLNs have significant physical stability, the system is also capable of the fabrication of easily degraded or highly sensitive drug molecules into solid lipid matrix and commendable *in vivo* acceptability by virtue of their biocompatibility [55].

Salamouni *et al*. investigated the effect of autoclaving on the physical stability of developed SLNs and nanostructured lipid carrier loaded with brimonidine. Autoclaving allowed the production of physically stable formulations with particle sizes below 500 nm. Moreover, the autoclaved samples appeared to be superior to non-autoclaved ones, due to their increased zeta potential values, indicating better physical stability, as well as an increased amount of brimonidine-base entrapped in the tested formulations [56].

SLNs loaded with methazolamide were prepared by a modified emulsion-solvent evaporation method and the potential as a new therapeutic system for glaucoma was evaluated. The developed SLNs provided appropriate particle size with better tolerability, good IOP-lowering capacity compared to commercial formulations in lower doses, and longer duration of IOP reduction [57]. Surface-modified SLNs containing timolol with and without phospholipids were formulated by melt emulsification with high-pressure homogenization. Higher encapsulation efficiency was obtained with surface-modified SLNs compared with unmodified SLNs, and high and sustained permeation were also achieved with surface modified SLN formulations compared with timolol solution [58].

5.1.3. Nanomicelles

Nanomicelles are nano-sized spherical vesicles comprising the hydrophobic core and hydrophilic shells capable of self-clustering in the aqueous phase at concentrations greater than that of critical micellar concentrations (CMCs). These systems are suitable for passive drug transport for hydrophobic drug moieties. The micellization process is stabilized by an aqueous medium, which is able to form an indefinable balance of intermolecular forces [59]. Micelle-based ocular delivery is a promising approach to treat ocular diseases because the nano-size range has properties, which improve

penetration of the transcorneal membrane and increase intraocular absorption, as well as minimize ocular irritation and tearing [60]. Additionally, these systems have numerous advantages including a sustained drug release pattern, diminished side effects, and the capability of loading hydrophobic drugs into clear aqueous solutions. The micellar systems have improved stability and precision, and avoid blurring of vision as compared to conventional dosage forms [61].

Pepic *et al*. developed triblock copolymer Pluronic F127-based nanomicelles with pilocarpine and evaluated the surface activity and miotic response on rabbit eyes. Developed nanomicelles showed improved pharmacokinetic parameters when compared to standard pilocarpine solutions. The best results were obtained for the nanomicelles prepared with pilocarpine, which exhibited significant prolongation of miotic activity and an increased area under the curve (AUC) to 64% [62]. Ribeiro *et al*. investigated the encapsulation and release of ethoxzolamide from single and mixed poloxamine micelles for topical ocular delivery. Mixed micelles showed higher solubilization capability, better physical stability, and more sustained drug release compared with single poloxamine micelles [63]. Pluronic-based metipranolol loaded micelles were prepared using ethyl acetate as the dispersion agent and coated with chitosan to improve bioavailability. Chitosan coated nanomicelles achieved suitable physicochemical characters for ophthalmic use including particle size, surface charge, morphology, turbidity, stability, and loading efficiency. Furthermore, the *in vitro* and *in vivo* studies indicated that these micelles showed sustained release behavior and good pharmaceutical responses [64]. Methoxy-poly(ethylene glycol)-b-poly(ε-caprolactone) polymeric micelles loaded with methazolamide were prepared using the thin film hydration procedure. The optimized formulation exhibited a particle size of 60 nm, spherical shape, 93% of entrapment efficiency, sustained drug release, and superior ocular tolerability. Moreover, a better *in vivo* inhibitory effect of developed polymeric micelles was achieved compared to drug solution on a glaucoma induced rabbit eye model [65].

5.1.4. Dendrimers

Dendrimers are nanostructured tree-like branched polymeric vehicles and have the potential for playing a significant role in nanomedicine drug development due to the important property of allowing an enormous ratio of multivalent surface molecules to molecular volume. Thus, dendrimers also are important for the development of ophthalmic drug delivery [66, 67]. The terminal branches of dendrimers have functional groups with different charges [68]. Each functional end group acts as a solubilizing agent, targeting moiety, or therapeutic molecule. The dendrimers mainly consist of two regions: the central portion of the moiety is specifically called the "core", and the peripheral chain terminals are referred to as "dendrons" [69]. As a consequence, three key elements can be clearly identified that can alter the confined nature to facilitate typical interactions with tissues: the extremely branched inner core; the replicate entity known as the level of generations; and terminal multivalent functional moieties [70, 71]. The dendrimer solution is easily spread on the aqueous layer of corneal tissues, and due to the tensioactive properties of the formulations, it effectively binds to the mucous layer of the cornea to improve ocular retention time [72].

Vandamme and Brobeck studied the influence of increasing the size and molecular weight and functional groups of different generations of poly(amidoamine) (PAMAM) dendrimers for ocular delivery. Ocular tolerance and retention on the ocular surface were evaluated after mixing with different generations of PAMAM dendrimers in the rabbit eye model. The same model was also used to determine the prolonged miotic or mydriatic activities of dendrimer solutions containing pilocarpine nitrate and tropicamide, respectively. The residence time was longer for the solutions containing dendrimers with carboxylic and hydroxyl surface groups. No pro-

longation of residence time occurred when increasing dendrimer concentration. The residence time of PAMAM dendrimer on the cornea showed size and molecular weight dependency [66].

DenTimol, a dendrimer-based polymeric timolol analog, was developed by a two steps reaction process. Briefly, a timolol precursor was reacted with the heterobifunctional amine polyethylene glycol (PEG) acetic acid *via* a ring-opening reaction of an epoxide by an amine to form the timolol-PEG conjugate. Timolol-PEG was then coupled to an ethylenediamine (EDA) core polyamidoamine (PAMAM) dendrimer to form DenTimol. DenTimol showed no signs of toxicity or ocular irritation. In addition, it was efficient at crossing the cornea and displayed an excellent IOP lowering effect [73]. Lancina *et al*. developed fast-dissolving dendrimer-based nanofibers loaded with brimonidine tartrate as a topical delivery system for glaucoma treatment. No toxicity was noted at therapeutic levels in cultured cells or ocular irritation in a rat model after the administration of developed nanofibers. Intra-ocular pressure response was equivalent between developed nanofibers and drug solution in a single dose test, but developed nanofibers showed improved efficacy with daily dosing over a 3-week test period. Disposition experiment showed developed nanofibers had the capacity to accumulate in the anterior chamber [74].

Acetazolamide loaded poly(propylene imine) dendrimers were synthesized using ethylendiamine as a dendrimer core through the divergent approach and their intraocular pressure-lowering potential was evaluated. The results suggested that, in addition to size, charge and molecular geometry, poly(propylene imine) dendrimers showed mucoadhesion and prolonged the ocular residence time which ultimately enhanced the ocular bioavailability of the incorporated acetazolamide compared to drug solutions [75]. Spataro *et al*. developed phosphorus-containing dentritic compounds possessing one quaternary ammonium salt as the core and carboxylic acid terminal groups loaded with carteolol as the ocular anti-hypertensive drug for the management of glaucoma. Carboxylic acid groups in dendrimers are able to interact with the amino function of carteolol, through electrostatic interactions. Generation 0 of the drug-dendrimer complex was soluble, whereas generation 1 and 2 were poorly soluble. Due to the very low solubility of generation 2, the amount of drug instilled was low, but the amount of drug that penetrated inside the eyes was 2.5 fold larger than the drug alone [76]. Table **1** illustrates recent studies on polymer-based nanocarrier systems for the treatment of glaucoma.

5.2. Vesicular Nanocarriers

5.2.1. Liposomes

Liposome encompasses large concentric spheres of lipid bilayers consisting of one or more spheres of lipid bilayers segregated by water chambers having a size ranging from 80 nm to 100 μm in diameter. Due to their amphiphilic character, liposomes can accommodate both lipophilic and hydrophilic drugs corresponding to the lipid bilayer and aqueous compartment, respectively. Liposomes are classified as small unilamellar vesicles (SUV) (10-100 nm) and large unilamellar vesicles (LUV) (100-300 nm) based on the size. If there is more than one bilayer they are generally referred to as multilamellar vesicles (MLV) [77]. Based on the lipid content, the liposomes have a positive, negative or neutral surface charge, which generally determines the retention time on corneal surfaces.

Li *et al*. investigated the potential of brinzolamide loaded liposomes as a topical ocular delivery system for local glaucoma therapy. The liposomes were produced by the thin-film dispersion method with a particle size of 84.33 ± 2.02 nm and entrapment efficiency of $98.32 \pm 1.61\%$. The *in vitro* release of brinzolamide from liposome was slower than drug suspension. *Ex vivo* corneal transport experiment indicated that liposomes had a 6.2-fold higher transmembrane permeation than drug suspension. Liposomes achieved ten times stronger efficacy in IOP reduction than drug suspension in white New Zealand rabbits [78].

Table 1.   Recent studies on polymer-based nanocarrier systems for the treatment of glaucoma.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Useful Findings
NANOPARTICLES				
Chitosan & derivatives	TPP	Ionic gelation	Timolol maleate	Drug release rate depended mainly on the used polymers, particle size of pre-pared nanocarriers and drug loading [50].
Chitosan	TPP	Ionic gelation	Carteolol	γ-Scintigraphy study showed good spread and retention in precorneal area as compared to the aqueous carteolol solution and prolonged reduction in intraocular pressure [51].
Chitosan and sodium aliginate.	TPP	Ionic gelation	Dorzolamide	Developed nanoparticles offers a more intensive treatment of glaucoma and a better patient compliance as it requires fewer applications per day compared to conventional eye drops [52].
Chitosan and sodium aliginate	Calcium chloride	Pre- ionic gelation method	Timolol maleate	The results indicated that the cornea penetration of drug loaded in nanoparticles was twice than that of plain drug [53].
PLGA	Poly vinyl alcohol, Vitamin E TPGS	Double emulsification - solvent evaporation technique	Dorzolamide	Nanoparticles with PVA and TPGS as emulsifiers significantly reduced the intraocular pressure by 22.81% and 29.12%, respectively, after a single topical instillation into the eye [54].
SLN				
Glyceryl monostearate	Castor oil	Modified high shear homogenization method.	Brimonidine	Autoclaving at 121°C for 15min allowed the production of physically stable formulations in nanometric range, below 500nm suitable for ophthalmic application [56].
Phospholipids (Lipoid S100)	Tween 80, Polyethylene glycol 400	Modified emulsion–solvent evaporation	Methazolamide	The developed SLN had higher therapeutic efficacy, later occurrence of maximum action, and more prolonged effect than drug solution and commercial product [57].
Phospholipon 90G	Sorbitol, Polysorbate 80, Theobroma oil	Melt emulsification with high pressure homogenization.	Timolol hydrogen maleate	Surface-modified SLN could provide an efficient way of improving ocular bioavailability of loaded drug [58].
NANOMICELLES				
Pluronic F127;	Water and Phosphate buffer.	Dissolution method	Pilocarpine	The best results were obtained for the micellar pilocarpine base solution which exhibits significant prolongation of miotic activity and an increase of AUC for 64% [62].
Poloxamine	0.9 % sodium chloride.	Direct dissolution method.	Ethoxzolamide	The different structural features of poloxamines and their combination in mixed micelles enabled the tuning of drug release profiles, sustaining the release in the 1-5 days range [63].
Pluronic	Chitosan	Dissolution method	Metipranolol	The *in vitro* and *in vivo* studies indicate the pluronic micelles modified by chitosan have sustained release behaviour and good pharmacological response [64].
Methoxy-poly(ethylene glycol)-b-poly(εcaprolactone) polymeric micelles	--	Thin film hydration procedure	Methazolamide	Drug loaded polymeric micelles showed sustained release, cellular and tissue biocompatibility and anti-glaucoma efficiency, as compared to drug solution [65].

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Useful Findings
DENDRIMERS				
PAMAM	-	-	Pilocarpine nitrate; Tropicamide	Residence time was longer for the solutions containing dendrimers with carboxylic and hydroxyl surface groups. The remanence time of PAMAM dendrimer solutions on the cornea showed size and molecular weight dependency [66].
PAMAM	Amine polyethylene glycol acetic acid	--	Timolol precursor	About 8% of the dendrimeric drug permeated through the cornea in 4 h. An IOP reduction by an average of 7.3 mmHg (~30% reduction from baseline) was observed [73].
PAMAM	Methoxy polyethylene glycol	--	Brimonidine tartrate	Intra-ocular pressure response was equivalent between dendrimeric drug solution and drug solution in a single dose test, but dendrimeric drug solution showed improved efficacy with daily dosing over a 3-week test period [74].
Poly(propylene imine)	Ethylenediamine	-	Acetazolamide	The sustained and prolonged reduction in intraocular pressure suggested that drug entrapped in dendrimers can be used for higher retention in ocular cul-de sac [75].

Key to abbreviations: TPP- Triphenyl Phosphate; PLGA- Polylactic-co-Glycolic Acid; PVA-Poly Vinyl Alcohol; TPGS- D -α-Tocopherol polyethylene glycol 1000 succinate; SLN – Solid Lipid Nanoparticle; PAMAM – Polyamidoamine; IOP-Intra Ocular Pressure; AUC – Area under curve

Reverse-phase evaporation liposomes (RELs) and multilamellar liposomes (MLLs) encapsulated with acetazolamide were prepared by using the reverse-phase evaporation and lipid film hydration methods consisting of egg phosphatidylcholine and cholesterol with or without stearylamine or dicetyl phosphate as positive and negative charge inducers, respectively. MLLs were larger in size than RELs and exhibited higher values of entrapment efficiencies. Drug loading was increased by increasing cholesterol content as well as by the inclusion of strarylamine. Drug release rates showed an order of negatively charged > neutral > positively charged liposomes, which is the reverse of the data of drug loading efficiency. MLLs produced a more significant lowering in IOP and showed a more sustained action than RELs because of the presence of several lipid bilayers that release the drug slowly over a prolonged period of time [79].

Brinzolamide loaded soybean phosphatidylcholine (S100) and cholesterol-based liposomes with or without TPGS were developed by a thin-film dispersion method. TPGS containing liposomes exhibited better stability, sustained drug release, enhanced trans-corneal transport, extended the cornea residence time, and maintained an effective IOP reduction when compared with normal liposomes and commercial drug products. Moreover, no eye irritation or lesions were observed in healthy white rabbits after administration of developed liposomes [80].

5.2.2. Niosomes

Niosomes are the main constituent of non-ionic surfactants; they are similar to that of bilayered liposome vesicles. The aqueous and lipid bilayer of niosomes can readily accommodate the hydrophilic and lipophilic nature of drug moieties [81]. Niosomes have numerous advantages over liposome vesicles such as high physical and chemical stability, less expense, nontoxic, and nonimmunogenic [82-84].

Aqueous humor concentration of timolol maleate in albino rabbits, after instillation of one drop of timolol maleate solution or a mucoadhesive coated niosomal system containing 0.25 % of timolol maleate was measured using the microdialysis method. The peak concentration of the drug in aqueous humor and AUC for niosomes was almost 1.7 times and 2.3 times higher than the control drug solution, respectively. An important observation was that the high drug concentrations achieved upon niosomes administration were maintained for up to 2 h [85].

Niosomes containing Span 60/cholesterol entrapped with acetazolamide dissolved in 7 % v/v PEG 400 were prepared using the thin-film hydration method. Changes in Span 60/cholesterol altered the percentage entrapment efficiency and drug release. The highest entrapment was obtained with multilamellar niosomes prepared from Span 60/cholesterol in a 4:2 molar ratio. The *in vivo* evaluation showed that acetazolamide niosomes were found to markedly decrease and most effectively prolong the decrease in the IOP in comparison to the free drug solution [86].

Hashim *et al*. prepared niosomal hydrogel containing 0.5% (w/v) atenolol for the treatment of glaucoma by the thin film hydration method using Span 60/cholesterol at different molar ratios and carbopol 934P as the gelling agent. Higher entrapment efficiency was obtained from niosomes prepared using Span 60/cholesterol at a 2: 1 molar ratio with a particle size diameter of 94± 8.1 nm. Niosomal hydrogel formulation using carbopol 934P significantly exhibited a sustained *in vitro* release of atenolol compared with free drug solution and other polymeric hydrogels. *In vivo* studies proved that niosomal hydrogel was found to show the most significant prolonged decrease in IOP compared with other atenolol formulations of the same concentration [87].

5.2.3. Cubosomes

Cubosomes, also known as liquid crystalline nanoparticles, were developed to overcome the limitation of liposomes instability. Cubosomes are distinct particles from disintegration and steric stabilization of cubic phases of lipids in excess amounts of water [88]. The microstructure of cubosomes looks like biological membranes [89]. Cubosomes have higher stability due to the strong electric repulsion and a large ratio of the lipid bilayer [90-92]. In addition, cubosomes have higher penetration, adhesion and excellent biocompatibility with the corneal surface. They can facilitate high drug

loading and sustained release properties owing to their enlarged interior surface area and cubic crystalline structure [93].

Timolol maleate cubosomes were prepared using glycerol monooleate and poloxamer 407 *via* high-pressure homogenization. The cubosomes with the Pn3m internal structure had a mean particle size of 142 nm and high encapsulation efficiency ($86.4 \pm 4.6\%$). *Ex vivo* and *in vivo* experiments revealed that the cubosomes were more capable of increasing the corneal penetration by prolonging the drug retention time and enhancing the IOP-lowering effect of Timolol maleate than the commercially available eye drops. Additionally, the cytotoxicity examination of rabbit corneal cells and histological evaluation revealed that cubosomes are biocompatible and show no toxicity [94]. Wu *et al.*, developed brinzolamide encapsulated cubosomes by a modified emulsification method as a therapeutic system for topical ocular delivery. Cubosomes displayed prolonged drug release and 3.47-fold increase in the permeability coefficient compared with that of Azopt® in *in vitro* and *ex vivo* release studies, respectively. Cubosomes provided better tolerability, equal IOP-lowering strength as Azopt® in lower doses, and longer duration of action [95]. Table **2** illustrates recent studies on vesicular based nanocarrier systems for the treatment of glaucoma.

5.3. Dispersed System

5.3.1. Microemulsions

The colloidal dispersion of microemulsions (MEs) is defined as minute oil globules dispersed into the aqueous phase with the help of suitable surfactant molecules. The system is thermodynamically stable, optically isotropic, and materializes as transparent solutions with the dispersion of globule size diameters of less than 200 nm. The dispersed system is used to improve the solubility of lipophilic drugs [96]. In addition, the characteristic properties include low surface tension, and hence excellent wetting and spreading ability on tissue surfaces. MEs can be appropriate as ocular drug delivery systems since they provide increased residence time, improves corneal penetration and prolonged drug release. Additionally, they can be delivered through the topical route as eye drops without creating visibility problems due to their nano droplet size. Moreover, they are economical and easy to technologically transfer to large scale productions [97].

Chan *et al.* developed phase transition microemuslion systems consisting of two non-ionic surfactants, sorbitan monolaurate and polyoxyethylene sorbitan mono-oleate with ethyl oleate as the oil component and different amounts of water (5%-100%) as the aqueous phase for ocular delivery of the hydrophilic drug pilocarpine hydrochloride. These systems undergo phase changes from ME to liquid crystalline and to the coarse emulsion with a change in viscosity depending on the water content. Drug release depended on the viscosity with lower release rates obtained from formulations with higher viscosity. The miotic response and duration of action were greatest in the case of microemulsion containing 5%, 10% (water/oil microemulsion) and 26% (liquid crystalline) of the aqueous phase [98].

For microemulsion-laden gels to be effective ocular topical drug delivery systems, these systems should load sufficient quantities of the drug and release the drug in a controlled manner. Li *et al.* developed ethyl butyrate entrapped in water as oil/water microemulsions stabilized by Pluronic F127 surfactant in 2-hydroxyethyl methacrylate (HEMA) gels, and measured the transport rates of timolol. Gels that had timolol loaded microemulsions exhibited a slow and extended drug release [99].

Timolol maleate loaded water/oil microemulsions were prepared by mixing ethyl oleate (oily phase) with polyoxyethylene 20 sorbitan monooleate and sorbitan monolaurate (surfactants) and water (aqueous phase), and evaluated their capacity to reduce the IOP of the glaucomatous rabbit eye model. A reduction in intraocular pressure was seen for 12 h compared to aqueous eye drops that lasted only 5 h. In addition, *ex vivo* permeation through goat cornea revealed the delayed release of Timolol maleate from microemulsion as compared with its aqueous solution [100].

Ince *et al.* prepared pilocarpine trapped microemulsion containing soybean oil as the oil phase, Brij 35P, and Span 80 as surfactants, 1-butanol as cosurfactant and water as the aqueous phase prepared by the titration method. The developed microemulsion showed good physicochemical properties and stability for six months. After microemulsion instillation into the rabbit eyes, the intraocular pressure was reduced significantly. The ocular irritation test suggests that the microemulsion formulation does not cause significant allergies to the eye [101].

5.3.2. Nanoemulsion

The nanoemulsion delivery system has three components including the aqueous phase, oily phase, and a combination of surface-active agents [102]. Nanoemulsions are defined as the nanometric size range of oil droplets dispersed into an external aqueous phase and stabilized by a suitable surfactant or cosurfactant system. This system offers good physical stability due to its droplet diameter of less than 1000 nm that is kinetically stable. This preparation needs small amounts of surfactant and high input energy for homogenization [103, 104].

Oil-in-water brinzolamide nanoemulsions were developed by the spontaneous emulsification method with triacetin and CapryolTM 90 (oil phase) with the use of various surfactants [Cremophor RH 40; Brij® 35; Labrasol® and tyloxapol; and Transcutol® P (as co-surfactant)] and water. In all nanoemulsions, the average size of the globules was found to be less than 40 nm. *In vitro* release studies indicated that the release efficiency in most of the nanoemulsions was high as compared to the drug suspension. The pharmacodynamic study revealed that nanoemulsions provide the same bioavailability as the commercially available suspension, even with lower drug content [105].

Morsi *et al.* formulated acetazolamide loaded nanoemulsions using peanut oil (oil phase), tween ®80 and/or cremophor®EL as the surfactant in addition to transcutol® P or propylene glycol as cosurfactants. Gellan gums alone and in combination with xanthan gum, HPMC or carbopol were mixed with nanoemulsions to obtain ion-induced nanoemulsion-based *in situ* gel systems. The nanoemulsion based *in situ* gels showed significantly sustained drug release in comparison to the nanoemulsion. Gellan/xanthan and gellan/HPMC showed higher therapeutic efficacy and more prolonged intraocular pressure-lowering effects relative to that of commercial eye drops and oral tablets. Gellan/xanthan showed superiority over gellan/HPMC in all studied parameters [106].

Nanoemulsions consisting isopropyl myristate as lipophilic disperse phase, Tween®80 and Lipoid®E80 as emulsifying agents, and water as an aqueous phase with plain timolo maleate or timolol maleate complexed with Bis-(2-ethylexyl)-sulfosuccinate were prepared. An increased drug permeation and accumulation into the corneas were observed using timolol maleate- Bis-(2-ethylexyl)-sulfosuccinate complex compared to plain timolo maleate due to an increase of lipophilicity of the drug as ion-pairs. The addition of thickening/mucoadhesive polymers significantly increases the viscosity of the preparation, which prolonged residence time on the preocular surface. The inclusion of chitosan in nanoemulsions led to a marked increase in drug permeation, probably due to interactions with the negative charges on ocular surfaces [107].

Eighteen acetazolamide nanoemulsions consisting of different oils, surfactants and cosurfactants at various ratios and constant water content (39% or 59%) were prepared based on their constructed pseudoternary-phase diagrams. The drug release rate from nanoemulsions was dependent on the type of surfactant and cosurfactant used in preparations. Nanoemulsions, which were prepared with higher water content (59%), exhibited faster drug release. Therapeutic efficacy testing revealed that nanoemulsion prepared

Table 2.   Recent studies on the vesicular based nanocarrier systems for the treatment of glaucoma.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Useful Findings
LIPOSOMES				
Soybean Phosphati-dylcholine (S100)	Cholesterol	Thin film dispersion method	Brinzolamide	Drug loaded liposome showed a more sustained and effective intraocular pressure reduction than drug suspension [78].
L-phosphatidylcholine,	Stearylamine, dice-tyl phosphate.	Reverse-phase evapo-ration method & lipid film Hydration meth-ods	Acetazolamide	The positively charged and neu-tral liposomes exhibited greater lowering in IOP and a more prolonged effect than the negatively liposomes [79].
TPGS	-	Thin-film dispersion method	Brinzolamide	The developed liposomes maintained an effective intraocular pressure reduction from 3 h to 11 h after administration, while drug suspension and normal liposomes did 3 h to 6 h and 3 h to 8 h respec-tively [80].
NIOSOMES				
Span 60	Cholesterol, Chito-san.	Reverse-phase evaporation method	Timolol maleate	Chitosan coated niosomes showed enhanced drug absorption which was almost 1.7 fold as compared to that of drug solution [85].
Span 60	Cholesterol, PEG 400	Thin film hydration technique	Acetazolamide	Changing the surfactant/cholesterol ratio altered the entrapment efficiency and drug release. The optimized niosomes considerably lowering of the IOP than free drug solution [86].
Span 60	Cholesterol, car-bopal 934P	-	Atenolol	Niosomal hydrogel formulation was found to show the most significant prolonged decrease in IOP compared with commercial eye drops [87].
CUBOSOMES				
Glycerol monooleate	Poloxamer 407	High pressure homog-enization technique	Timolol Maleate	Reduced the IOP in rabbits from 27.8~39.7 to 21.4~32.6 mmHg after 1-week administration and had a longer retention time and better lower-IOP effect than the marketed eye drops [94].
Glycerol monooleate	Poloxamer 407	Modified emulsifica-tion	Brinzolamide	Prolonged drug release and 3.47-fold increase perme-ability coefficient compared with that of Azopt® [95].

Key to abbreviations: TPGS- D -α-Tocopherol polyethylene glycol 1000 succinate; PEG- Polyethylene glycol; IOP-Intra Ocular Pressure

with peanut oil, Tween 80, Cremophor EL, Transcutol P and 39% water content showed better and prolonged IOP lowering effects relative to either commercial acetazolamide eye drops (Azopt®) or the commercial oral acetazolamide tablet (Cidamex®) [108].

Poloxamer-based thermoreversible dorzolamide hydrochloride *in situ* gel nanoemulsion were prepared for ocular topical delivery. The optimized formulation, consisting of Triacetin (7.80%), Polox-amer 407 (13.65%), Poloxamer 188 (3.41%), Miranol C2M (4.55%), and water (70.59%), enhanced the therapeutic efficacy of this drug relative to either simple drug solution or the market drug product. The insertion of poloxamer 407 in this nanoemulsion showed a superior pharmacodynamic activity and longer residence in the eye due to thermoreversible gelation [109]. Table **3** illustrates recent studies on the dispersed system based nanocarrier systems for the treatment of glaucoma.

6. POSITIVELY CHARGED NANOCARRIERS

Cornea and conjunctiva have a negative charge on their surface, due to the presence of negatively charged mucus residues on the outer side of their membranes and due to selective active ion pumps [110]. Ocular surfaces should thus be selective for positively charged delivery systems that interact with cells, leading to in-creased drug permeability and prolonged pharmacological effects (Fig. **2**) [111]. The use of nanotechnology provides attractive op-portunities for ocular drug delivery, mainly because the association of an active molecule to a nanocarrier allows the molecule to inter-act intimately with specific ocular structures, and thus overcome ocular barriers and prolong drug residence in the target tissue. Fur-thermore, this technology offers a promising solution for formulat-ing various poorly water-soluble drugs in the form of eye drops. Recent progress in nanocarrier science has led to the usage of nanomicelles, nanoemulsion, liposomes, and nanocapsules as the vehicle for controlled and targeted delivery of drugs. The advan-tages are a small size, long half-life, high stability, bioavailability, reduced dosing frequency, reduced side-effects, and improved pa-tient compliance. Moreover, the efficiency of drug loading and delivery is influenced by the physicochemical properties such as
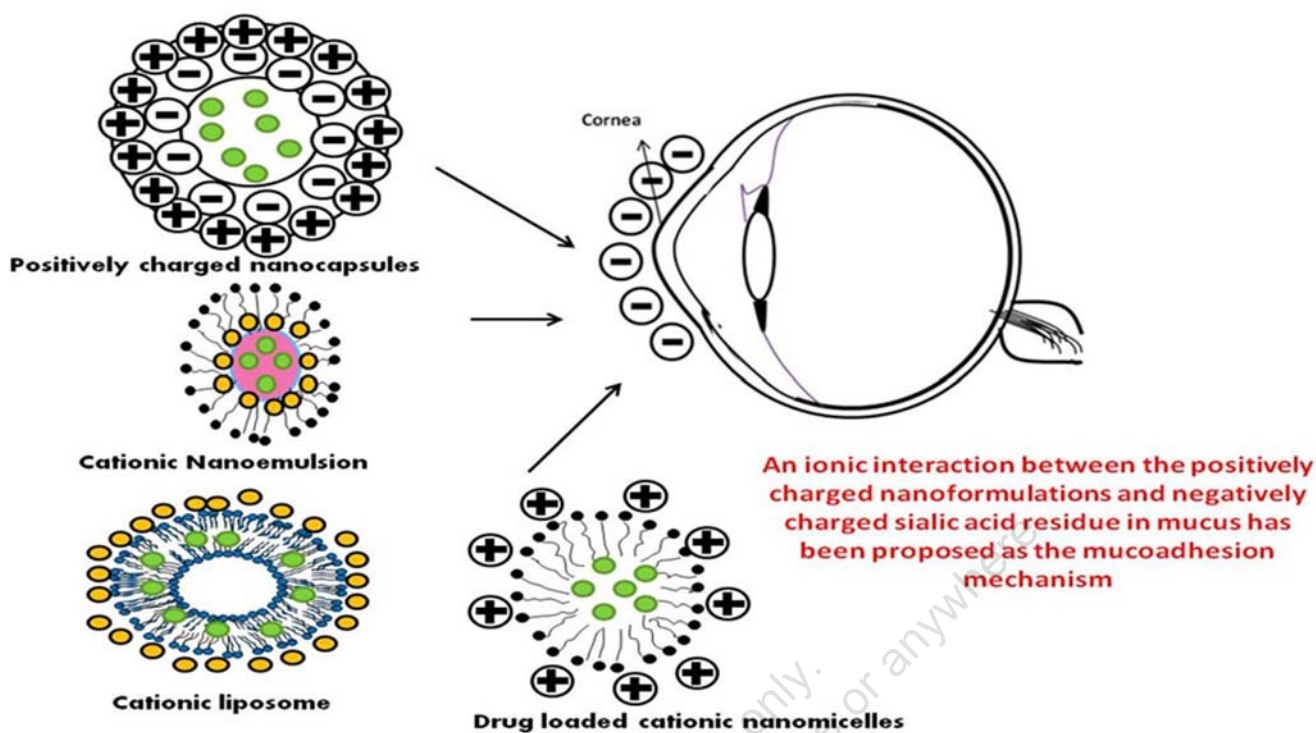
Fig. (2). Diagrammatic representation of positively charged nanocarriers for ocular drug delivery. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

size, composition, surface area and surface charge of the carriers, which in turn affects the bioavailability of the drugs [112, 113].

For the past decade, increasing attention has been paid to delivery systems fabricated from natural biopolymer-based polyelectrolyte complexes (PEC), formed by electrostatic interactions between two oppositely charged biopolymers [114]. The preparation of nanocapsules *via* the complex coacervation method is based on the electrostatic interactions between cationic and anionic polymers, resulting in the formulation of insoluble spherical beads or capsules. In many cases, the complexation of polyelectrolytes is performed in an aqueous solution, allowing the encapsulation of biological components, including enzymes or peptides, as their biological activities can be retained. Chitosan, as a unique positively charged polysaccharide, has been one of the most popular biopolymers for the development of drug delivery systems for various applications, due to its promising properties including high biocompatibility, excellent biodegradability, and low toxicity, as well as abundant availability and low production cost. Due to the protonation of amino groups on the backbone, chitosan becomes a cationic polyelectrolyte in acidic medium, which can form PEC with negatively charged polyelectrolytes, allowing various applications.

In recent years, positively charged liposomes and nanoemulsions have been used as drug carriers. Positively charged liposomes prepared with stearylamine, a cationic lipid, have been used for ocular delivery of acyclovir [110]. Ocular mucoadhesive chitosan-coated liposome formulations have exhibited higher retention and improved ocular bioavailability of ciprofloxacin hydrochloride [115]. Chitosan is suitable for the formulation of mucoadhesive cationic nanoemulsions because it is positively charged, making it able to adhere to the negatively charged oil globules in oil/water nanoemulsions. Furthermore, chitosan is soluble in diverse acids and able to interact with polyanions to form complexes and nanogels. The cornea and conjunctiva have negative charges so the mucoadhesive polymer might interact intimately with these structures and increase the concentration and residence time of the associated drug at the disease site. An ionic interaction between the positively charged amino groups of chitosan and negatively charged sialic acid residues in mucus has been proposed as the mucoadhesion mechanism.

D- α-Tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS or TPGS) is a derivative of the natural vitamin E (α-tocopherol), which is conjugated with PEG 1000 [116]. The lipophilic portion of TPGS allows poorly soluble drugs to be solubilized in the nanomicelles, which may lead to more effective treatment for ocular therapy. TPGS on acidification (*i.e.* TPGS-COOH) is available for conjugation to proteins and macromolecules. This property of TPGS has been exploited for the delivery of many therapeutic agents using nanomedicines. Further, conjugation of chitosan with TPGS can (i) increase solubility at acidic pH environments, (ii) decrease burst release of the encapsulated drug, (iii) increase stability of drug, (iv) reverse zeta potential promoting cellular adhesion and retention of the delivery system at the site, and (v) enhance permeation by the reversible opening of tight junctions [117, 118].

Melatonin loaded cationic SLN were developed using Softisan® as a lipid matrix along with two lipid modifiers (stearic acid and palmitic acid) and didecyldimethiammonium bromide as a cationic lipid. The authors concluded that nanocarriers containing stearic acid were the most successful in terms of IOP reduction, and the effects lasted approximately 24 h [119]. Methazolamide loaded solid lipid nanoparticles were prepared with or without chitosan by the modified emulsion solvent evaporation method. The addition of chitosan in SLN produced positive zeta potential, high physical stability, enhancement of transcorneal permeation and absence of cytotoxicity *in vivo* compared to SLN without chitosan. *In vivo* observation revealed that the prepared chitosan-coated SLN maintained the reduction of IOP levels for prolonged periods of time when compared to that of plain SLN and commercial drug solution [120].

Table 3. Recent studies on dispersed systems based nanocarriers for the treatment of glaucoma.

OIL	Other Component (Surfactant and co Surfactant)	Fabrication Method	Drug Encapsulated	Useful Findings
MICROEMULSION				
Ethyl oleate	Tween 80, span 20.	Water titration method	Pilocarpine hydrochloride	Drug release depended on the viscosity with lower release rates obtained from formulations with high viscosity. The miotic response and duration of action were greatest in case of microemulsions indicating high ocular bioavailability [98].
Ethyl butyrate	Pluronic F127 and sodium caprylate.	Water titration method	Timolol maleate	Gels that had timolol loaded microemulsions exhibited a slow and extended drug release in distilled water. All of the gels exhibited a very rapid release in PBS and in saline due to higher solubility of timolol in these solutions compared to that in distilled water [99].
Ethyl oleate	Tween 80,Span 20	Water titration method	Timolol maleate	A reduction in intraocular pressure was seen lasting for 12 h compared to aqueous eye drop that lasted for only 5 h [100].
Soybean oil	Brij 35P, Span 80, and 1-butanol.	Water titration method	Pilocarpine	Developed formulation had satisfying stability and low dose applications caused no irritation and no pathological effects on the eye and decreased the IOP in glaucoma rabbit model [101].
NANOEMULSION				
Triacetin and Capryol™ 90,	Cremophor RH 40, Brij 35, Labrasol, tyloxapol and Transcutol P	Spontaneous emulsification method.	Brinzolamide	The result showed that optimized nanoemulsions have able to reduce the Intra ocular pressure and *in vitro* release efficiency was superior as compared to brinzolamide suspension [105].
Peanut oil	Tween 80 and cremophore EL and transcutol P	Water titration method	Acetazolamide	In situ gelling system revealed that higher therapeutic efficiency and more prolonged intraocular pressure reducing effect was achieved [106].
Soya oil (capryc-caprylic triglyceride)	Soya lecithin, Tween80, and Pluronic® F68	High pressure homogenization.	Timolol maleate	Nanoemulsion containing timolol maleate or Hydrophobic ion pairing of timolol with AOT is able to enhance the corneal permeation and accumulation of drug both in solutions and emulsions [107].
Isopropyl myristate, oleic acid, peanut oil	Cremophor EL, Tween80,transcutol P and water.	Water titration method	Acetazolamide	Prolonged intraocular pressure lowering effect was observed as compared to commercial available brinzolamide eye drops [108].
Triacetin	Poloxamer 407 , Poloxamer 188, Miranol C2M and water	Water titration method	Dorzolamide hydrochloride	In situ gel nanoemulsion showed superior therapeutic efficacy as compared to drug solution or commercially available dorzolamide hydrochloride eye drops [109].

Key to abbreviations: IOP-Intra Ocular Pressure; AOT- Bis-(2-ethylexyl)-sulfosuccinate

Lutfi and Muzeyyen prepared positively-charged pilocarpine HCl-loaded polymeric and lipid nanoparticles used by Eudragit(®) RS 100, Gelucire(®) 44/14 and octadecylamine as a cationic agent through the quasi-emulsion solvent evaporation technique. The formulations were evaluated for different physicochemical properties and stability studies for 6 months. The authors suggested that nanoparticles were more stable in lyophilized form than aqueous solution form for long-term storage [121]. Recently, we have reported brinzolamide loaded positively charged chitosan pectin mucoadhesive nanocapsules prepared by the polyelectrolyte complex coacervation method. The developed nanocapsules showed longer precorneal retention time, and sustained release of loaded brinzola-

mide and greater intraocular pressure-lowering effects were attained when compared to commercially available drug products [122].

CONCLUSION

Effective management of glaucoma is a major challenge because of the presence of ocular barriers. Consequently, the design of carrier systems with improved drug delivery properties to the ocular surface would be a promising step towards the management of glaucoma. Nanocarriers have emerged in the field of nanomedicine for topical ocular delivery to facilitate higher intraocular bioavailability of therapeutic agents. The topical ocular delivery of nanocarriers is a new avenue for the viable and enhanced manage-

ment of glaucoma. The development of nanocarriers using different excipients should be considered because even small changes in pH or temperature can interfere with the stability of the formulation. Cautious and radical selection of the excipients for ocular drug delivery is an additional major concern. Polymers which are biodegradable and biocompatible, with non-toxic profiles should be used. The development of positively charged nanocarrier-based ocular drug delivery systems is of intense interest because of prolonged drug retention times and improved drug penetration rates through electrostatic interaction on ocular surfaces. Recent advances in topical ocular delivery systems have been achieved with the expansion of a large range of nanocarriers with diverse properties including cationic nanoparticles, liposomes, dendrimers, nanoemulsion, and peptides. This review highlights the potential of a diverse array of nanocarriers as novel delivery platforms for efficient treatment of glaucoma.

FUTURE DIRECTION

Treating ocular diseases, and in particular glaucoma, with gene therapy will change the treatment of the disease from a general to a more personalized and efficient treatment that could improve the lives of many affected people. With the advent of efficient genome editing tools, it is now reasonable to think that the genomic component of glaucoma can be targeted for therapeutic and prophylactic purposes. The success of gene therapy relies on the efficient delivery of the genetic material to target cells, achieving optimum long-term gene expression. Although viral vectors have been widely used, their potential risks including immunogenicity and mutagenesis have promoted the design of non-viral vectors. Non-viral gene delivery systems are an extensive class of man-made complexes composed of a nucleic acid cargo, typically a plasmid, with various soft matters such as lipids, surfactants, biological and synthetic polymers [123].

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated systems (Cas9) represent powerful tools for studying diseases through the creation of model organisms generated by targeted modification and by the correction of disease mutations for therapeutic purposes [124]. Recently, advances in nonviral delivery systems have been made with the development of a vast range of nano-/microcarriers with diversities in targeting property modifications. Among them, cationic nanocarriers, formed by positively charged lipids or polymers, are most commonly used in gene delivery as nonviral vectors, because the positively charged surface could load and condense nucleic acids simply by electrostatic interactions [125]. With this background, there is little doubt that advances in the field of genome editing and nanotechnology will translate these methods to glaucoma therapy in the near future.

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REFERENCES

[1]   Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90(3): 262-7.
http://dx.doi.org/10.1136/bjo.2005.081224 PMID: 16488940

[2]   Cholkar K, Trinh HM, Pal D, Mitra AK. Discovery of novel inhibitors for the treatment of glaucoma. Expert Opin Drug Discov 2015; 10(3): 293-313.
http://dx.doi.org/10.1517/17460441.2015.1000857   PMID: 25575654

[3]   Motwani SK, Chopra S, Talegaonkar S, Kohli K, Ahmad FJ, Khar RK. Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimisation and *in vitro* characterisation. Eur J Pharm Biopharm 2008; 68(3): 513-25.
PMID: 17983737

[4]   Alonso MJ. Nanomedicines for overcoming biological barriers. Biomed Pharmacother 2004; 58(3): 168-72.
http://dx.doi.org/10.1016/j.biopha.2004.01.007 PMID: 15082339

[5]   Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems--recent advances. Prog Retin Eye Res 1998; 17(1): 33-58.
http://dx.doi.org/10.1016/S1350-9462(97)00002-5 PMID: 9537794

[6]   Kesavan K, Nath G, Pandit J. Preparation and *in vitro* antibacterial evaluation of gatifloxacin mucoadhesive gellan system. Daru 2010; 18(4): 237-46.
PMID: 22615622

[7]   Mansour M, Mansour S, Mortada ND, Abd Elhady SS. Ocular poloxamer-based ciprofloxacin hydrochloride in situ forming gels. Drug Dev Ind Pharm 2008; 34(7): 744-52.
http://dx.doi.org/10.1080/03639040801926030 PMID: 18612913

[8]   Kesavan K, Kant S, Singh PN, Pandit JK. Effect of hydroxypropyl-β-cyclodextrin on the ocular bioavailability of dexamethasone from a pH-induced mucoadhesive hydrogel. Curr Eye Res 2011; 36(10): 918-29.
http://dx.doi.org/10.3109/02713683.2011.593728 PMID: 21950697

[9]   Champalal KD, Sushilkumar P. Current status of ophthalmic *in-situ* forming hydrogel. Int J Pharma Bio Sci 2012; 3: 372-88.

[10]   Almeida H, Amaral MH, Lobão P, Sousa Lobo JM. Applications of poloxamers in ophthalmic pharmaceutical formulations: an overview. Expert Opin Drug Deliv 2013; 10(9): 1223-37.
http://dx.doi.org/10.1517/17425247.2013.796360 PMID: 23688342

[11]   Barar J, Asadi M, Mortazavi-Tabatabaei SA, Omidi Y. Ocular drug delivery; impact of *in vitro* cell culture models. J Ophthalmic Vis Res 2009; 4(4): 238-52.
PMID: 23198080

[12]   Yi X, Wang Y, Yu FS. Corneal epithelial tight junctions and their response to lipopolysaccharide challenge. Invest Ophthalmol Vis Sci 2000; 41(13): 4093-100.
PMID: 11095601

[13]   Ahmed I. The non corneal route in ocular drug delivery. Ophthalmic drug delivery systems. New York: Marcel Dekker 1993; 356-85.

[14]   Hämäläinen KM, Kananen K, Auriola S, Kontturi K, Urtti A. Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera. Invest Ophthalmol Vis Sci 1997; 38(3): 627-34.
PMID: 9071216

[15]   Kim SH, Lutz RJ, Wang NS, Robinson MR. Transport barriers in transscleral drug delivery for retinal diseases. Ophthalmic Res 2007; 39(5): 244-54.
http://dx.doi.org/10.1159/000108117 PMID: 17851264

[16]   Lara F, Bernal-Molina P, Fernández-Sánchez V, López-Gil N. Changes in the objective amplitude of accommodation with pupil size. Optom Vis Sci 2014; 91(10): 1215-20.
http://dx.doi.org/10.1097/OPX.0000000000000383   PMID: 25207484

[17]   Adhi M, Brewer E, Waheed NK, Duker JS. Analysis of morphological features and vascular layers of choroid in diabetic retinopathy using spectral-domain optical coherence tomography. JAMA Ophthalmol 2013; 131(10): 1267-74.
http://dx.doi.org/10.1001/jamaophthalmol.2013.4321   PMID: 23907153

[18]   Achouri D, Alhanout K, Piccerelle P, Andrieu V. Recent advances in ocular drug delivery. Drug Dev Ind Pharm 2013; 39(11): 1599-617.
http://dx.doi.org/10.3109/03639045.2012.736515 PMID: 23153114

[19]   Stewart PA, Tuor UI. Blood-eye barriers in the rat: correlation of ultrastructure with function. J Comp Neurol 1994; 340(4): 566-76.
http://dx.doi.org/10.1002/cne.903400409 PMID: 8006217

[20]   Shell JW. Pharmacokinetics of topically applied ophthalmic drugs. Surv Ophthalmol 1982; 26(4): 207-18.
http://dx.doi.org/10.1016/0039-6257(82)90081-9 PMID: 7041308

[21]   King-Smith PE, Fink BA, Nichols JJ, Nichols KK, Braun RJ, McFadden GB. The contribution of lipid layer movement to tear film thinning and breakup. Invest Ophthalmol Vis Sci 2009; 50(6): 2747-56.
http://dx.doi.org/10.1167/iovs.08-2459 PMID: 19218611

[22]   Garreis F, Gottschalt M, Schlorf T, *et al.* Expression and regulation of antimicrobial peptide psoriasin (S100A7) at the ocular surface and in the lacrimal apparatus. Invest Ophthalmol Vis Sci 2011; 52(7): 4914-22.
http://dx.doi.org/10.1167/iovs.10-6598 PMID: 21551409

[23]   Wilson CG, Zhu YP, Kurmala P, Rao LS, Dhillon B. Ophthalmic drug delivery. Drug delivery and targeting for pharmacists and pharmaceutical scientists. Boca Raton, Florida: CRC press 2001; 329-54.

[24]   Maïssa C, Guillon M. Tear film dynamics and lipid layer characteristics--effect of age and gender. Cont Lens Anterior Eye 2010; 33(4): 176-82.
http://dx.doi.org/10.1016/j.clae.2010.02.003 PMID: 20202891

[25]   Agrahari V, Mandal A, Agrahari V, *et al.* A comprehensive insight on ocular pharmacokinetics. Drug Deliv Transl Res 2016; 6(6): 735-54.
http://dx.doi.org/10.1007/s13346-016-0339-2 PMID: 27798766

[26]   Edward A, Prausnitz MR. Predicted permeability of the cornea to topical drugs. Pharm Res 2001; 18(11): 1497-508.
http://dx.doi.org/10.1023/A:1013061926851 PMID: 11758755

[27]   Huang AJ, Tseng SC, Kenyon KR. Paracellular permeability of corneal and conjunctival epithelia. Invest Ophthalmol Vis Sci 1989; 30(4): 684-9.
PMID: 2703309

[28]   Freddo TF. Shifting the paradigm of the blood-aqueous barrier. Exp Eye Res 2001; 73(5): 581-92.
http://dx.doi.org/10.1006/exer.2001.1056 PMID: 11747359

[29]   Barar J, Javadzadeh AR, Omidi Y. Ocular novel drug delivery: impacts of membranes and barriers. Expert Opin Drug Deliv 2008; 5(5): 567-81.
http://dx.doi.org/10.1517/17425247.5.5.567 PMID: 18491982

[30]   Kamei M, Misono K, Lewis H. A study of the ability of tissue plasminogen activator to diffuse into the subretinal space after intravitreal injection in rabbits. Am J Ophthalmol 1999; 128(6): 739-46.
http://dx.doi.org/10.1016/S0002-9394(99)00239-1 PMID: 10612511

[31]   Chen MS, Hou PK, Tai TY, *et al.* Blood-ocular barriers. Tzu-Chi Med J 2008; 20: 25-34.
http://dx.doi.org/10.1016/S1016-3190(08)60004-X

[32]   Runkle EA, Antonetti DA. The blood-retinal barrier: structure and functional significance. Methods Mol Biol 2011; 686: 133-48.
http://dx.doi.org/10.1007/978-1-60761-938-3_5 PMID: 21082369

[33]   Bouhenni RA, Dunmire J, Sewell A, Edward DP. Animal models of glaucoma. J Biomed Biotechnol 2012; 2012: 692609.
http://dx.doi.org/10.1155/2012/692609 PMID: 22665989

[34]   Salomão SR, Mitsuhiro MR, Belfort R Jr. Visual impairment and blindness: an overview of prevalence and causes in Brazil. An Acad Bras Cienc 2009; 81(3): 539-49.
http://dx.doi.org/10.1590/S0001-37652009000300017 PMID: 19722022

[35]   Franca JR, Foureaux G, Fuscaldi LL, *et al.* Bimatoprost-loaded ocular inserts as sustained release drug delivery systems for glaucoma treatment: *in vitro* and *in vivo* evaluation. PLoS One 2014; 9(4): e95461.
http://dx.doi.org/10.1371/journal.pone.0095461 PMID: 24788066

[36]   Jay JL, Allan D. The benefit of early trabeculectomy *versus* conventional management in primary open angle glaucoma relative to severity of disease. Eye (Lond) 1989; 3(Pt 5): 528-35.

http://dx.doi.org/10.1038/eye.1989.84 PMID: 2698360

[37]   Flammer J, Orgül S, Costa VP, *et al.* The impact of ocular blood flow in glaucoma. Prog Retin Eye Res 2002; 21(4): 359-93.
http://dx.doi.org/10.1016/S1350-9462(02)00008-3 PMID: 12150988

[38]   Satilmis M, Orgül S, Doubler B, Flammer J. Rate of progression of glaucoma correlates with retrobulbar circulation and intraocular pressure. Am J Ophthalmol 2003; 135(5): 664-9.
http://dx.doi.org/10.1016/S0002-9394(02)02156-6 PMID: 12719074

[39]   McAlinden C. Selective laser trabeculoplasty (SLT) *vs* other treatment modalities for glaucoma: systematic review. Eye (Lond) 2014; 28(3): 249-58.
http://dx.doi.org/10.1038/eye.2013.267 PMID: 24310236

[40]   Yadav KS, Rajpurohit R, Sharma S. Glaucoma: Current treatment and impact of advanced drug delivery systems. Life Sci 2019; 221: 362-76.
http://dx.doi.org/10.1016/j.lfs.2019.02.029 PMID: 30797820

[41]   Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. JAMA 2014; 311(18): 1901-11.
http://dx.doi.org/10.1001/jama.2014.3192 PMID: 24825645

[42]   Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. Br J Ophthalmol 2002; 86(2): 238-42.
http://dx.doi.org/10.1136/bjo.86.2.238 PMID: 11815354

[43]   Geyer O, Loewenstein A, Shalmon B, Neudorfer M, Lazar M. The additive miotic effects of dapiprazole and pilocarpine. Graefes Arch Clin Exp Ophthalmol 1995; 233(7): 448-51.
http://dx.doi.org/10.1007/BF00180951 PMID: 7557512

[44]   De S, Robinson D. Polymer relationships during preparation of chitosan-alginate and poly-l-lysine-alginate nanospheres. J Control Release 2003; 89(1): 101-12.
http://dx.doi.org/10.1016/S0168-3659(03)00098-1 PMID: 12695066

[45]   Chaurasia SS, Lim RR, Lakshminarayanan R, Mohan RR. Nanomedicine approaches for corneal diseases. J Funct Biomater 2015; 6(2): 277-98.
http://dx.doi.org/10.3390/jfb6020277 PMID: 25941990

[46]   Kreuter J. Nanoparticles as bioadhesive ocular drug delivery systems. Bioadhesive drug delivery systems. Boca Raton, Florida: CRC Press 1990; 203-12.

[47]   Mainardes RM, Silva LP. Drug delivery systems: past, present, and future. Curr Drug Targets 2004; 5(5): 449-55.
http://dx.doi.org/10.2174/1389450043345407 PMID: 15216911

[48]   Ding S. Recent developments in ophthalmic drug delivery. Pharm Sci Technol Today 1998; 8: 328-35.
http://dx.doi.org/10.1016/S1461-5347(98)00087-X

[49]   Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloid surf B 2010; 75: 1-18.
http://dx.doi.org/10.1016/j.colsurfb.2009.09.001

[50]   Siafaka PI, Titopoulou A, Koukaras EN, *et al.* Chitosan derivatives as effective nanocarriers for ocular release of timolol drug. Int J Pharm 2015; 495(1): 249-64.
http://dx.doi.org/10.1016/j.ijpharm.2015.08.100 PMID: 26341322

[51]   Ameeduzzafar , Ali J, Bhatnagar A, Kumar N, Ali A. Chitosan nanoparticles amplify the ocular hypotensive effect of cateolol in rabbits. Int J Biol Macromol 2014; 65: 479-91.
http://dx.doi.org/10.1016/j.ijbiomac.2014.02.002 PMID: 24530326

[52]   Katiyar S, Pandit J, Mondal RS, *et al. In situ* gelling dorzolamide loaded chitosan nanoparticles for the treatment of glaucoma. Carbohydr Polym 2014; 102: 117-24.
http://dx.doi.org/10.1016/j.carbpol.2013.10.079 PMID: 24507263

[53]   Ilka R, Mohseni M, Kianirad M, Naseripour M, Ashtari K, Mehravi B. Nanogel-based natural polymers as smart carriers for the controlled delivery of Timolol Maleate through the cornea for glaucoma. Int J Biol Macromol 2018; 109: 955-62.
http://dx.doi.org/10.1016/j.ijbiomac.2017.11.090 PMID: 29154878

[54]   Warsi MH, Anwar M, Garg V, *et al.* Dorzolamide-loaded PLGA/vitamin E TPGS nanoparticles for glaucoma therapy: Pharmacoscintigraphy study and evaluation of extended ocular hypotensive effect in rabbits. Colloids Surf B Biointerfaces 2014; 122: 423-31.
http://dx.doi.org/10.1016/j.colsurfb.2014.07.004 PMID: 25159319

[55]   Muller RH. Solid lipid nanoparticles (SLN) - an alternative colloidal carrier system for controlled drug delivery. Eur J Biopharm 1995; 41: 62-9.

[56]   El-Salamouni NS, Farid RM, El-Kamel AH, El-Gamal SS. Effect of sterilization on the physical stability of brimonidine-loaded solid lipid nanoparticles and nanostructured lipid carriers. Int J Pharm 2015; 496(2): 976-83.
http://dx.doi.org/10.1016/j.ijpharm.2015.10.043 PMID: 26498372

[57]   Li R, Jiang S, Liu D, *et al.* A potential new therapeutic system for glaucoma: solid lipid nanoparticles containing methazolamide. J Microencapsul 2011; 28(2): 134-41.
http://dx.doi.org/10.3109/02652048.2010.539304 PMID: 21142697

[58]   Attama AA, Reichl S, Müller-Goymann CC. Sustained release and permeation of timolol from surface-modified solid lipid nanoparticles through bioengineered human cornea. Curr Eye Res 2009; 34(8): 698-705.
http://dx.doi.org/10.1080/02713680903017500 PMID: 19899997

[59]   Lu Y, Park K. Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. Int J Pharm 2013; 453(1): 198-214.
http://dx.doi.org/10.1016/j.ijpharm.2012.08.042 PMID: 22944304

[60]   Li J, Tian S, Tao Q, *et al.* Montmorillonite/chitosan nanoparticles as a novel controlled-release topical ophthalmic delivery system for the treatment of glaucoma. Int J Nanomedicine 2018; 13: 3975-87.
http://dx.doi.org/10.2147/IJN.S162306 PMID: 30022821

[61]   Mandal A, Bisht R, Rupenthal ID, Mitra AK. Polymeric micelles for ocular drug delivery: From structural frameworks to recent preclinical studies. J Control Release 2017; 248: 96-116.
http://dx.doi.org/10.1016/j.jconrel.2017.01.012 PMID: 28087407

[62]   Pepić I, Jalsenjak N, Jalsenjak I. Micellar solutions of triblock copolymer surfactants with pilocarpine. Int J Pharm 2004; 272(1-2): 57-64.
http://dx.doi.org/10.1016/j.ijpharm.2003.11.032 PMID: 15019069

[63]   Ribeiro A, Sosnik A, Chiappetta DA, Veiga F, Concheiro A, Alvarez-Lorenzo C. Single and mixed poloxamine micelles as nanocarriers for solubilization and sustained release of ethoxzolamide for topical glaucoma therapy. J R Soc Interface 2012; 9(74): 2059-69.
http://dx.doi.org/10.1098/rsif.2012.0102 PMID: 22491977

[64]   Lin HR, Chang PC. Novel pluronic-chitosan micelle as an ocular delivery system. J Biomed Mater Res B Appl Biomater 2013; 101(5): 689-99.
http://dx.doi.org/10.1002/jbm.b.32871 PMID: 23359519

[65]   Elmowafy E, Gad H, Biondo F, Casettari L, Soliman ME. Exploring optimized methoxy poly(ethylene glycol)-block-poly(ε-caprolactone) crystalline cored micelles in anti-glaucoma pharmacotherapy. Int J Pharm 2019; 566: 573-84.
http://dx.doi.org/10.1016/j.ijpharm.2019.06.011 PMID: 31176850

[66]   Vandamme TF, Brobeck L. Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. J Control Release 2005; 102(1): 23-38.
http://dx.doi.org/10.1016/j.jconrel.2004.09.015 PMID: 15653131

[67]   Xu Q, Kambhampati SP, Kannan RM. Nanotechnology approaches for ocular drug delivery. Middle East Afr J Ophthalmol 2013; 20(1): 26-37.
http://dx.doi.org/10.4103/0974-9233.106384 PMID: 23580849

[68]   Kompella UB, Amrite AC, Pacha Ravi R, Durazo SA. Nanomedicines for back of the eye drug delivery, gene delivery, and imaging. Prog Retin Eye Res 2013; 36: 172-98.
http://dx.doi.org/10.1016/j.preteyeres.2013.04.001 PMID: 23603534

[69]   Honda M, Asai T, Oku N, Araki Y, Tanaka M, Ebihara N. Liposomes and nanotechnology in drug development: focus on ocular targets. Int J Nanomedicine 2013; 8: 495-503.
http://dx.doi.org/10.2147/IJN.S30725 PMID: 23439842

[70]   Chaplot SP, Rupenthal ID. Dendrimers for gene delivery--a potential approach for ocular therapy? J Pharm Pharmacol 2014; 66(4): 542-56.
http://dx.doi.org/10.1111/jphp.12104 PMID: 24635556

[71]   Rodríguez Villanueva J, Navarro MG, Rodríguez Villanueva L. Dendrimers as a promising tool in ocular therapeutics: Latest advances and perspectives. Int J Pharm 2016; 511(1): 359-66.
http://dx.doi.org/10.1016/j.ijpharm.2016.07.031 PMID: 27436708

[72]   Bravo-Osuna I, Vicario-de-la-Torre M, Andrés-Guerrero V, *et al.* Novel water-soluble mucoadhesive carbosilane dendrimers for ocular administration. Mol Pharm 2016; 13(9): 2966-76.
http://dx.doi.org/10.1021/acs.molpharmaceut.6b00182 PMID: 27149661

[73]   Lancina MG III, Wang J, Williamson GS, Yang H. DenTimol as a dendrimeric timolol analogue for glaucoma therapy: Synthesis and preliminary efficacy and safety assessment. Mol Pharm 2018; 15(7): 2883-9.
http://dx.doi.org/10.1021/acs.molpharmaceut.8b00401 PMID: 29767982

[74]   Lancina MG III, Singh S, Kompella UB, Husain S, Yang H. Fast dissolving dendrimer nanofiber mats as alternative to eye drops for more efficient antiglaucoma drug delivery. ACS Biomater Sci Eng 2017; 3(8): 1861-8.
http://dx.doi.org/10.1021/acsbiomaterials.7b00319 PMID: 29152562

[75]   Mishra V, Jain NK. Acetazolamide encapsulated dendritic nano-architectures for effective glaucoma management in rabbits. Int J Pharm 2014; 461(1-2): 380-90.
http://dx.doi.org/10.1016/j.ijpharm.2013.11.043 PMID: 24291772

[76]   Spataro G, Malecaze F, Turrin CO, *et al.* Designing dendrimers for ocular drug delivery. Eur J Med Chem 2010; 45(1): 326-34.
http://dx.doi.org/10.1016/j.ejmech.2009.10.017 PMID: 19889480

[77]   Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chem Rev 2015; 115(19): 10938-66.
http://dx.doi.org/10.1021/acs.chemrev.5b00046 PMID: 26010257

[78]   Li H, Liu Y, Zhang Y, *et al.* Liposomes as a novel ocular delivery system for brinzolamide: *in vitro* and *in vivo* studies. AAPS PharmSciTech 2016; 17(3): 710-7.
http://dx.doi.org/10.1208/s12249-015-0382-1 PMID: 26335415

[79]   Hathout RM, Mansour S, Mortada ND, Guinedi AS. Liposomes as an ocular delivery system for acetazolamide: *in vitro* and *in vivo* studies. AAPS PharmSciTech 2007; 8(1): 1-12.
http://dx.doi.org/10.1208/pt0801001 PMID: 17408209

[80]   Jin Q, Li H, Jin Z, *et al.* TPGS modified nanoliposomes as an effective ocular delivery system to treat glaucoma. Int J Pharm 2018; 553(1-2): 21-8.
http://dx.doi.org/10.1016/j.ijpharm.2018.10.033 PMID: 30316795

[81]   Carafa M, Santucci E, Alhaique F, *et al.* Preparation and properties of new unilamellar non-ionic/ionic surfactant vesicles. Int J Pharm 1998; 160: 51-9.
http://dx.doi.org/10.1016/S0378-5173(97)00294-9

[82]   Kaur IP, Garg A, Singla AK, Aggarwal D. Vesicular systems in ocular drug delivery: an overview. Int J Pharm 2004; 269(1): 1-14.
http://dx.doi.org/10.1016/j.ijpharm.2003.09.016 PMID: 14698571

[83]   Saettone MF, Perini G, Carafa M, *et al.* Non-ionic surfactant vesicles as ophthalmic carriers for cyclopentolate. A preliminary evaluation. STP Pharma Sci 1996; 6: 94-8.

[84]   Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm 1998; 172: 33-70.
http://dx.doi.org/10.1016/S0378-5173(98)00169-0

[85]   Kaur IP, Aggarwal D, Singh H, Kakkar S. Improved ocular absorption kinetics of timolol maleate loaded into a bioadhesive niosomal delivery system. Graefes Arch Clin Exp Ophthalmol 2010; 248(10): 1467-72.
http://dx.doi.org/10.1007/s00417-010-1383-0 PMID: 20437246

[86]   El-Menshawe SF. A novel approach to topical acetazolamide/PEG 400 ocular niosomes. J Drug Deliv Sci Technol 2012; 22: 295-9.
http://dx.doi.org/10.1016/S1773-2247(12)50049-3

[87]   Abu Hashim II, El-Dahan MS, Yusif RM, Abd-Elgawad AE, Arima H. Potential use of niosomal hydrogel as an ocular delivery system for atenolol. Biol Pharm Bull 2014; 37(4): 541-51.
http://dx.doi.org/10.1248/bpb.b13-00724 PMID: 24694602

[88]   Rizwan SB, McBurney WT, Young K, *et al.* Cubosomes containing the adjuvants imiquimod and monophosphoryl lipid A stimulate robust cellular and humoral immune responses. J Control Release 2013; 165(1): 16-21.
http://dx.doi.org/10.1016/j.jconrel.2012.10.020 PMID: 23142776

[89]   Zhang J, Wang S. Topical use of coenzyme Q10-loaded liposomes coated with trimethyl chitosan: tolerance, precorneal retention and anti-cataract effect. Int J Pharm 2009; 372(1-2): 66-75.
http://dx.doi.org/10.1016/j.ijpharm.2009.01.001 PMID: 19437594

[90]   Salonen A, Moitzi C, Salentinig S, Glatter O. Material transfer in cubosome-emulsion mixtures: effect of alkane chain length. Langmuir 2010; 26(13): 10670-6.
http://dx.doi.org/10.1021/la100955z PMID: 20443641

[91]   Nguyen TH, Hanley T, Porter CJ, Boyd BJ. Nanostructured liquid crystalline particles provide long duration sustained-release effect for a poorly water soluble drug after oral administration. J Control Release 2011; 153(2): 180-6.
http://dx.doi.org/10.1016/j.jconrel.2011.03.033 PMID: 21497623

[92]   Gordon S, Young K, Wilson R, *et al.* Chitosan hydrogels containing liposomes and cubosomes as particulate sustained release vaccine delivery systems. J Liposome Res 2012; 22(3): 193-204.
http://dx.doi.org/10.3109/08982104.2011.637502 PMID: 22188610

[93]   Karami Z, Hamidi M. Cubosomes: remarkable drug delivery potential. Drug Discov Today 2016; 21(5): 789-801.
http://dx.doi.org/10.1016/j.drudis.2016.01.004 PMID: 26780385

[94]   Huang J, Peng T, Li Y, *et al.* Ocular cubosomes drug delivery system for timolol maleate: preparation, characterization, cytotoxicity, *ex vivo*, and *in vivo* evaluation. AAPS PharmSciTech 2017; 18(8): 2919-26.
http://dx.doi.org/10.1208/s12249-017-0763-8 PMID: 28429294

[95]   Wu W, Li J, Wu L, *et al.* Ophthalmic delivery of brinzolamide by liquid crystalline nanoparticles: *in vitro* and *in vivo* evaluation. AAPS PharmSciTech 2013; 14(3): 1063-71.
http://dx.doi.org/10.1208/s12249-013-9997-2 PMID: 23813437

[96]   Schmalfuss U, Neubert R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Release 1997; 46: 279-85.
http://dx.doi.org/10.1016/S0168-3659(96)01609-4

[97]   Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv drug deliv 2000; 45: 89-102.
http://dx.doi.org/10.1016/S0169-409X(00)00103-4

[98]   Chan J, Maghraby GM, Craig JP, Alany RG. Phase transition water-in-oil microemulsions as ocular drug delivery systems: *in vitro* and *in vivo* evaluation. Int J Pharm 2007; 328(1): 65-71.
http://dx.doi.org/10.1016/j.ijpharm.2006.10.004 PMID: 17092668

[99]   Li CC, Abrahamson M, Kapoor Y, Chauhan A. Timolol transport from microemulsions trapped in HEMA gels. J Colloid Interface Sci 2007; 315(1): 297-306.
http://dx.doi.org/10.1016/j.jcis.2007.06.054 PMID: 17673246

[100]   Hegde RR, Bhattacharya SS, Verma A, Ghosh A. Physicochemical and pharmacological investigation of water/oil microemulsion of non-selective beta blocker for treatment of glaucoma. Curr Eye Res 2014; 39(2): 155-63.
http://dx.doi.org/10.3109/02713683.2013.833630 PMID: 24073659

[101]   Ince I, Karasulu E, Ates H, *et al.* A novel Pilocarpine microemulsion as an ocular delivery system: *in vitro* and *in vivo* studies. J Clin Exp Ophthalmol 2015; 6: 408.

[102]   Chen H, Khemtong C, Yang X, Chang X, Gao J. Nanonization strategies for poorly water-soluble drugs. Drug Discov Today 2011; 16(7-8): 354-60.
http://dx.doi.org/10.1016/j.drudis.2010.02.009 PMID: 20206289

[103]   Delmas T, Piraux H, Couffin AC, *et al.* How to prepare and stabilize very small nanoemulsions. Langmuir 2011; 27(5): 1683-92.
http://dx.doi.org/10.1021/la104221q PMID: 21226496

[104]   Sharma B, Sharma A. Future prospect of nanotechnology in development of anti-ageing formulations. Int J Pharm Pharm Sci 2012; 4: 57-66.

[105]   Mahboobian MM, Foroutan SM, Aboofazeli R. Brinzolamide-loaded nanoemulsions: *in vitro* release evaluation. Iran J Pharm 2016; 12: 75-93.

[106]   Morsi N, Ibrahim M, Refai H, El Sorogy H. Nanoemulsion-based electrolyte triggered *in situ* gel for ocular delivery of acetazolamide. Eur J Pharm Sci 2017; 104: 302-14.
http://dx.doi.org/10.1016/j.ejps.2017.04.013 PMID: 28433750

[107]   Gallarate M, Chirio D, Bussano R, *et al.* Development of O/W nanoemulsions for ophthalmic administration of timolol. Int J Pharm 2013; 440(2): 126-34.
http://dx.doi.org/10.1016/j.ijpharm.2012.10.015 PMID: 23078859

[108]   Morsi NM, Mohamed MI, Refai H, *et al.* Nanoemulsion as a novel ophthalmic delivery system for acetazolamide. Int J Pharma Sci 2014; 6: 227-36.

[109]   Ammar HO, Salama HA, Ghorab M, Mahmoud AA. Development of dorzolamide hydrochloride *in situ* gel nanoemulsion for ocular delivery. Drug Dev Ind Pharm 2010; 36(11): 1330-9.
http://dx.doi.org/10.3109/03639041003801885 PMID: 20545523

[110]   Law SL, Huang KJ, Chiang CH. Acyclovir-containing liposomes for potential ocular delivery. Corneal penetration and absorption. J Control Release 2000; 63(1-2): 135-40.
http://dx.doi.org/10.1016/S0168-3659(99)00192-3 PMID: 10640587

[111]   Peira E, Carlotti ME, Trotta C, Cavalli R, Trotta M. Positively charged microemulsions for topical application. Int J Pharm 2008; 346(1-2): 119-23.
http://dx.doi.org/10.1016/j.ijpharm.2007.05.065 PMID: 17618070

[112]   Wadhwa S, Paliwal R, Paliwal SR, Vyas SP. Nanocarriers in ocular drug delivery: an update review. Curr Pharm Des 2009; 15(23): 2724-50.
http://dx.doi.org/10.2174/138161209788923886 PMID: 19689343

[113]   Diebold Y, Calonge M. Applications of nanoparticles in ophthalmology. Prog Retin Eye Res 2010; 29(6): 596-609.
http://dx.doi.org/10.1016/j.preteyeres.2010.08.002 PMID: 20826225

[114]   Luo Y, Wang Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. Int J Biol Macromol 2014; 64: 353-67.
http://dx.doi.org/10.1016/j.ijbiomac.2013.12.017 PMID: 24360899

[115]   Abdelbary G. Ocular ciprofloxacin hydrochloride mucoadhesive chitosan-coated liposomes. Pharm Dev Technol 2011; 16(1): 44-56.
http://dx.doi.org/10.3109/10837450903479988 PMID: 20025433

[116]   Kutty RV, Chia SL, Setyawati MI, Muthu MS, Feng SS, Leong DT. *In vivo* and *ex vivo* proofs of concept that cetuximab conjugated vitamin E TPGS micelles increases efficacy of delivered docetaxel against triple negative breast cancer. Biomaterials 2015; 63: 58-69.
http://dx.doi.org/10.1016/j.biomaterials.2015.06.005 PMID: 26081868

[117]   Guo Y, Chu M, Tan S, *et al.* Chitosan-g-TPGS nanoparticles for anticancer drug delivery and overcoming multidrug resistance. Mol Pharm 2014; 11(1): 59-70.
http://dx.doi.org/10.1021/mp400514t PMID: 24229050

[118]   Agrawal P, Sonali, Singh RP, *et al.* Bioadhesive micelles of d-α-tocopherol polyethylene glycol succinate 1000: Synergism of chitosan and transferrin in targeted drug delivery. Colloids Surf B Biointerfaces 2017; 152: 277-88.
http://dx.doi.org/10.1016/j.colsurfb.2017.01.021 PMID: 28122295

[119]   Leonardi A, Bucolo C, Drago F, Salomone S, Pignatello R. Cationic solid lipid nanoparticles enhance ocular hypotensive effect of melatonin in rabbit. Int J Pharm 2015; 478(1): 180-6.
http://dx.doi.org/10.1016/j.ijpharm.2014.11.032 PMID: 25448580

[120]   Wang F, Chen L, Zhang D, *et al.* Methazolamide-loaded solid lipid nanoparticles modified with low-molecular weight chitosan for the treatment of glaucoma: vitro and vivo study. J Drug Target 2014; 22(9): 849-58.
http://dx.doi.org/10.3109/1061186X.2014.939983 PMID: 25045926

[121]   Lütfi G, Müzeyyen D. Preparation and characterization of polymeric and lipid nanoparticles of pilocarpine HCl for ocular application. Pharm Dev Technol 2013; 18(3): 701-9.
http://dx.doi.org/10.3109/10837450.2012.705298 PMID: 22813238

[122]   Dubey V, Mohan P, Dangi JS, Kesavan K. Brinzolamide loaded chitosan-pectin mucoadhesive nanocapsules for management of glaucoma: Formulation, characterization and pharmacodynamic study. Int J Biol Macromol 2020; 152: 1224-32.
http://dx.doi.org/10.1016/j.ijbiomac.2019.10.219 PMID: 31751741

[123]   Oliveira AV, Rosa da Costa AM, Silva GA. Non-viral strategies for ocular gene delivery. Mater Sci Eng C 2017; 77: 1275-89.
http://dx.doi.org/10.1016/j.msec.2017.04.068 PMID: 28532005

[124]   Jain A, Zode G, Kasetti RB, *et al.* CRISPR-Cas9-based treatment of myocilin-associated glaucoma. Proc Natl Acad Sci USA 2017; 114(42): 11199-204.
http://dx.doi.org/10.1073/pnas.1706193114 PMID: 28973933

[125]   Li L, Hu S, Chen X. Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities. Biomaterials 2018; 171: 207-18.
http://dx.doi.org/10.1016/j.biomaterials.2018.04.031 PMID: 29704747