Expert Opinion

Ocular drug delivery – a look towards nanobioadhesives

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Importance of the field: A major challenge emanating in the design of topical ophthalmic preparations is their short precorneal residence time. Retention of a drug delivery system in the front of the eye is thus desirable. One solution identified to address this concern is a retentive system that can preferably be delivered in a liquid drop form and ultimately remain attached to the corneal tissue owing to incorporation of a bioadhesive component. Forward-thinking approaches are required to achieve advancements in this approach for the attainment of an effective drug concentration at the site of action. Accordingly, several investigators have identified the benefits of nanotechnology-based drug delivery systems for ophthalmic drug delivery.
Areas covered in this review: A concerted effort was made to review critically all 'nanobioadhesives', that is, nanosystems designed for ocular drug delivery with the goal of attaining prolonged ocular retention, in a systematic, chronological manner, from their reported point of inception to the present.
What the reader will gain: A perspective on possible future trends in this growing field of ocular drug delivery is formulated.
Take home message: The importance of and need for new developments in the field of ocular nanobioadhesives is emphasized.

Keywords: bioadhesive, colloidal carriers, liposome, mucoadhesive, nanocapsule, nanoparticle, nanosphere, ophthalmic drug delivery

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1. Introduction

The eye – a precious, unique and anatomically isolated specialized sensory organ – shows unique pharmacodynamic and pharmacokinetic properties. This is a consequence of its seclusion from systemic access by the blood–retinal, blood–aqueous and blood–vitreous barriers. Significantly, the eye can be viewed as an apt 'laboratory' for the investigation of drug delivery in inflammatory and infectious afflictions. According to Robinson [1] 'no other organ is so readily accessible or as visible for observation'; undoubtedly a distinct test for the pharmaceutical scientist in drug delivery system design.

The eye is divided into anterior and posterior segments, which function both independently and in tandem on application of an ocular preparation [2]. Targeting the drug to the appropriate site of action in the eye is usually one of the greatest challenges in drug delivery because of its anatomical and physiological defense mechanisms. Furthermore, most of the biodisposition studies in ocular drug delivery have been carried out using animal models, and the physiological and anatomical variation of the eye among species highlights that outcomes may not be directly applicable to human use. Ocular drug delivery systems thus necessitate specified criteria according to the physiological structure of the eye [3,4].

Ocular therapeutic preparations are centuries old and comprise topical ophthalmic solutions, suspensions and semisolids, which hold the disadvantage of

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Article highlights.

- The significance of nanotechnology to ocular drug delivery and the concept of nanobioadhesives are elaborated on.
- The ocular structures coming into contact with topically administered nanobioadhesive systems are highlighted. The polymers used in the design of ocular bioadhesive systems are introduced.
- Nanoparticles are defined, and their applicability to topical ocular delivery is exemplified.
- Nanoparticulate bioadhesive systems are reviewed in the sequence in which they were developed.
- Liposomes and niosomes are defined, and their applicability to topical ocular delivery is exemplified.
- Liposomal and niosomal bioadhesive systems are reviewed in the sequence in which they were developed.
- Recent innovations in ocular nanobioadhesive technologies are reviewed, including new chitosan-based therapies, cubosomes, approaches to achieve receptor-mediated bioadhesion and bioadhesive dendrimers.
- The reviewed nanobioadhesive systems are summarized and their proposed suitablility assessed.
- The authors' thoughts on nanobioadhesives are provided. Specifically, the potential toxicity of nanobioadhesives, modeling of nanobioadhesion, and forward-thinking approaches that could be considered in the design of ocular nanobioadhesive systems are discussed.

This box summarizes key points contained in the article.

extremely low bioavailability. Short precorneal residence time owing to the tear turnover, rapid nasolachrymal drainage of the instilled drug from the tear fluid and non-productive absorption through the conjunctiva results in very limited absorption of the drug through the lipophilic corneal barrier and absorption through the conjunctiva, which may result in an unwanted systemic absorption of the drug where local ocular action is required. Often only 1 – 10% or less of the dose reaches the anterior segment tissues of the eye; subsequently, only a fraction of the absorbed dose can partition to the posterior tissues. Frequent instillations are often required to achieve the required therapeutic effect, and this leads to escalating inconvenience and adverse effects. Should penetration occur, drug residence time and the ultimate duration of action are short. Numerous approaches have therefore been applied in the past few decades in an attempt to improve the precorneal residence time [3,4].

Retention of a drug delivery system in the front of the eye is thus desirable given the enormous loss of an instilled drug solution that typically occurs. Two solutions are evident: i) the use of erodible or non-erodible ocular inserts; or ii) a retentive system that can preferably be delivered in a liquid drop form. The former hold the disadvantage of poor tolerance by the patient; the latter are polymer-based, and responsively increase in viscosity on exposure to a stimulus (temperature, pH, ion or chemical) or remain attached to the precorneal tissue owing to incorporation of a bioadhesive component – this approach is easy to conceptualize but ultimately quite difficult to put into practice [5]. The value of viscosity enhancers and gels for prolonging precorneal residence time has only a limited value, because such liquid formulations are eliminated by the usual routes in the ocular domain [6].

Forward-thinking approaches are required to address these challenges in the treatment of ocular diseases. The goal in ocular therapeutics is to maintain an effective drug concentration at the site of action for an appropriate period of time, in order to achieve the expected pharmacological response [7]. Accordingly, several investigators have stated that, 'ophthalmic drug delivery, probably more than any other route of administration, may benefit from the characteristics of nanotechnology-based drug delivery systems' [8,9]. Nanosystems have thus been conceptualized and fabricated to circumvent the problems associated with conventional systems. The use of nanosystems/colloidal carriers is purported to provide numerous advantages for topical ocular drug delivery, because of their ability to protect the encapsulated molecule while facilitating its transport to the different compartments of the eye [10-16] as well as providing targeted delivery of bioactives. Furthermore, nanosystems can provide controlled drug delivery for extended periods of time – an attractive benefit for the treatment of some chronic ocular diseases [16-18] – and are purportedly patient-friendly, which is of utmost significance where use of the drug preparation by the patient is lifelong, for example, glaucoma [6,19]. Significantly, with future developments firmly in mind, nanosystems are paramount to exploitation of the emerging field of new gene therapies for the treatment of ocular disorders [9,20,21]. These colloidal carriers may be applied in liquid form in the same manner as eye drop solutions. By interaction with the glycoproteins of the cornea and conjunctiva they can form a precorneal depot, resulting in a prolonged release of the bound drug [22].

The main colloidal carriers under scrutiny for ocular delivery owing to their favorably small size include liposomes and nanoparticles [6]. Dendrimers are also emerging as a new ocular drug delivery form. The last two decades have thus seen directed efforts in the rational design of ocular drug delivery colloidal carriers/nanosystems. The starting point in the ocular drug delivery field was the development of hydrophobic nanosystems consisting of polyalkylcyanoacrylate [23-25] and polyesters, particularly poly-ε-caprolactone [26-28] as well as poly(lactic-*co*-glycolic acid) (PLGA) [29], highlighting the affinity or bioadhesive potential of these systems for the corneal epithelium owing to an appropriate surface charge or zeta potential [17], but also emphasizing their unattractive propensity to aggregate on contact with the mucosal surface. Thereafter, the aim was to create nanosystems with a hydrophilic coating with the purpose of improving their stability and their interaction with the mucosa. This was

achieved via optimization of ocular drug delivery nanosystems to obtain prolonged bioadhesion/residence time by the so-called 'mucoadhesive concept', based on entrapment of particles in the ocular mucus layer and interaction of bioadhesive polymer chains with mucins. Application of this generally results in a significant increase in precorneal residence time of the preparation [29,30]. Hydrophilic materials such as polyethyleneglycol (PEG) and chitosan [31,32] were selected because of their protein-rejecting properties (shielding effect), and mucoadhesive and penetration-enhancing properties and good biocompatibility with ocular structures, respectively [33-36]. De la Fuente *et al.* [9] elaborated on the evolution in the design of these nanosystems, with specific reference to chitosan (**Figure 1**).

Retention of the designed nanosystems in the ocular cul-de-sac following topical application is thus pivotal to achieve sustained drug release and prolonged therapeutic activity. The following considerations are paramount [6].

- Rapid leaching of drug from the particles is not conducive to sustained drug release; conversely, the concentration of the drug in the tears may be too low to attain adequate drug penetration into ocular tissues [37].
- It is a requisite that particle size for ophthalmic applications be within the nano-range to avoid irritation and foreign body sensation. Specifically, particles should be < 10 μm for eventual clearance through the lachrymal canals, which are 300 – 500 μm in diameter [38].
- Fabrication of particles from bioadhesive materials is essential for effective retention in ocular cul-de-sac. In the absence of bioadhesion, nanoparticle elimination from the precorneal site occurs as rapidly as an aqueous solution [6].

Several reviews have already succinctly delved into the concept of macroscopic topical ophthalmic solution, suspension, or semisolid bioadhesive systems; and the concept of a polymer that adheres to a mucous membrane is well established; however, bioadhesive systems based on nanotechnological approaches for ocular drug delivery have slipped under the radar over the past few years and have not been the main focus. A concerted effort was thus made to review critically all 'nanobioadhesives', that is, nanosystems designed for ocular drug delivery with the goal of attaining prolonged ocular retention, in a systematic, *chronological* manner, from their reported point of inception to the present. The concept of ocular bioadhesion is also elaborated on. Conclusively, a perspective on possible future trends in this growing field of ocular drug delivery is formulated.

2. Anatomy and physiology of the ocular surface and the concept of bioadhesion as embodied by polymers

This review is concerned with attachment of a bioadhesive nanosytem to the ocular surface, primarily the ocular mucins. The anatomical and physiological composition thereof is thus of primary interest. The structures of the eye, which come in contact with topically administered drug delivery systems, thus need a mention. The major refractive structure of the eye is the cornea [39]. The cornea is a clear, transparent, avascular tissue to which nutrients and oxygen are supplied by the lachrymal fluid and aqueous humor. It is composed of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium. The conjunctiva is a thin transparent membrane, which lines the inner surface of the eyelids and is reflected onto the globe. At the corneal margin, it is structurally continuous with the corneal epithelium. The membrane is vascular and moistened by the tear film. The goblet cells are an important anatomical element of the conjunctiva [30].

The ocular MUC mucins are implicated in forming the mucus layer of the tear film, in regulating tear film spread, and in inhibiting the adhesion of pathogens to the ocular surface. The mucins are produced by at least four of the *MUC* genes produced by the ocular surface.

- *MUC1*: MUC1 mucin products are omnipresent in the membranes of mucosal epithelia.
- *MUC2* and *MUC5AC*: produce secreted mucins and are present in the goblet cells of the conjunctiva. The trefoil factors or peptides (TFF1 and TFF3) also secreted by the goblet cells contribute to the rheological properties of the tear film by specific non-convalent interactions with mucins forming an entangled network [30].
- *MUC4*: the mucin produced by MUC4 has both membrane and secretory potential and has also been isolated from the lachrymal gland [40,41].

The degree and nature of glycosylation depend on the origin and ultimate functionality of the mucin. Compared with many gastrointestinal mucins, ocular mucins possess short oligosaccharide side chains, but carbohydrates typically comprise 60 – 80% dry weight of these mucins. Of pertinence here is the charge, which is always negative, that is conferred on the polymer by sialic acids and sulfate groups within these side chains [42,43]. The biochemically specific moieties that mucins possess impart binding functionality as well as a steric stiffness to the polypeptide core [41].

The concept of bioadhesion is not a new one, dictated by the propensity of cells to attach to each other with great strength. Ocular bioadhesion, specifically, refers to the capacity of certain polymers to adhere to the mucus coat covering the conjunctival and corneal surfaces of the eye by non-covalent bonds. The clearance time of bioadhesive polymeric systems is retarded as its dependence is shifted to mucus turnover rate rather than tear turnover rate. The importance of bioadhesive polymers lies in their ability to improve significantly the performance of controlled delivery systems through enhancement of drug delivery by means of optimum contact with the absorbing surface. Bioadhesive polymers, which consist of macromolecular hydrocolloids with numerous hydrophilic

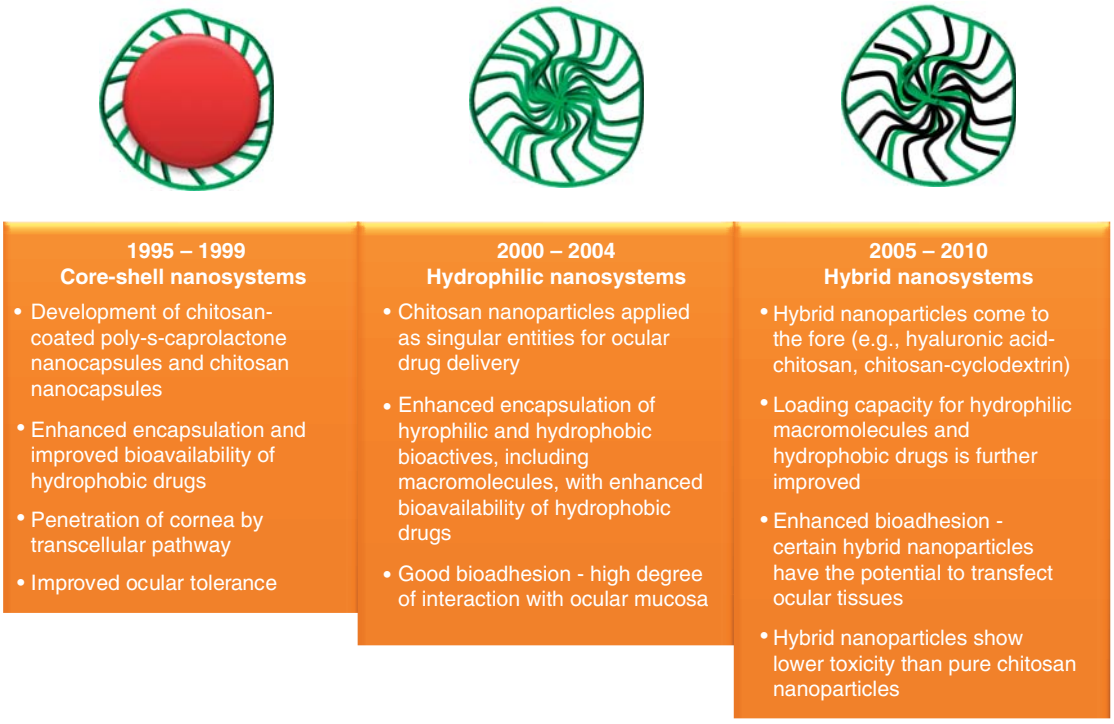

Figure 1. Time course highlighting the progression in design of nanosystems for ocular drug delivery, with a focus on the application of chitosan.
Adapted from [9].

(carboxyl-, hydroxyl-, amide-, sulfate-) functional groups, have found a pivotal role in ophthalmic drug delivery, their underlying mechanism of retention being attachment to the precorneal/conjunctival mucin layer via non-covalent bonds, and remaining in place for as long as the mucin is present. This ultimately prolongs the residence of the ocular dosage form in the cul-de-sac, which reduces dosing frequency [38,44]. **Figure 2** depicts the bioadhesive concept at the ocular surface, using a model nanosystem.

Diverse bioadhesive polymers have been implicated in the design of ocular nanosystems, each with their own merits. Polymers that have been subject to evaluation as adjuvants in ophthalmic drug delivery usually consist of macromolecular hydrocolloids with numerous hydrophilic functional groups, such as polyacrylic acid, carboxymethylcellulose and sodium hyaluronate (hyaluronic acid [HA]), which attach to the precorneal mucin layer by means of non-covalent bonds [45]. For example, inspiration was drawn from the actual composition of the eye to identify suitable polymeric candidates. Hyaluronic acid is a high-molecular-mass biological biopolymer composed of linear polysaccharides present in the vitreous body of the eye and in low concentrations in the aqueous humor [46]. Investigations have demonstrated its potency as a mucoadhesive polymer [47]. This is purportedly owing to its topical pseudoplastic properties, which result in improved protection of the cornea [44,46,48].

Some of the polymers implicated in the design of bioadhesive drug delivery systems are composed of synthetic mucoadhesive high-molecular-mass molecules (5000 – 10,000 Da) that cannot cross biological membranes, and include water-soluble polymers (linear chains) and water-insoluble polymers (swellable networks joined by crosslinking agents). Such molecules include cellulosic components such as sodium carboxyrnethylcellulose, or polyanion bioadhesives such as polyacrylic acid [44].

When looking at the properties contributing to the acceptability of a polymer for ocular drug delivery, the viscosity must be considered, which is a measure of its resistance to flow, originating from an interplay between its molecular mass, concentration, temperature and shear stress. Ultimately, the type of flow may be classified as Newtonian (viscosity independent of the shear rate) or non-Newtonian (viscosity changes with the shear rate). Newtonian systems demonstrate no real improvement of bioavailability above a certain viscosity, at which point there is no further increase of the residence contact time and blinking becomes painful with increasingly viscous gels [49-51]. Moreover, the viscosity of the preparation can obstruct the puncti and the canaliculi [45]. However, non-Newtonian formulations possessing pseudoplastic properties show a viscosity decrease with increasing shear rate during blinking (reaching rates of 10,000 s^{-1}) and ocular movement, thus exerting significantly less resistance to

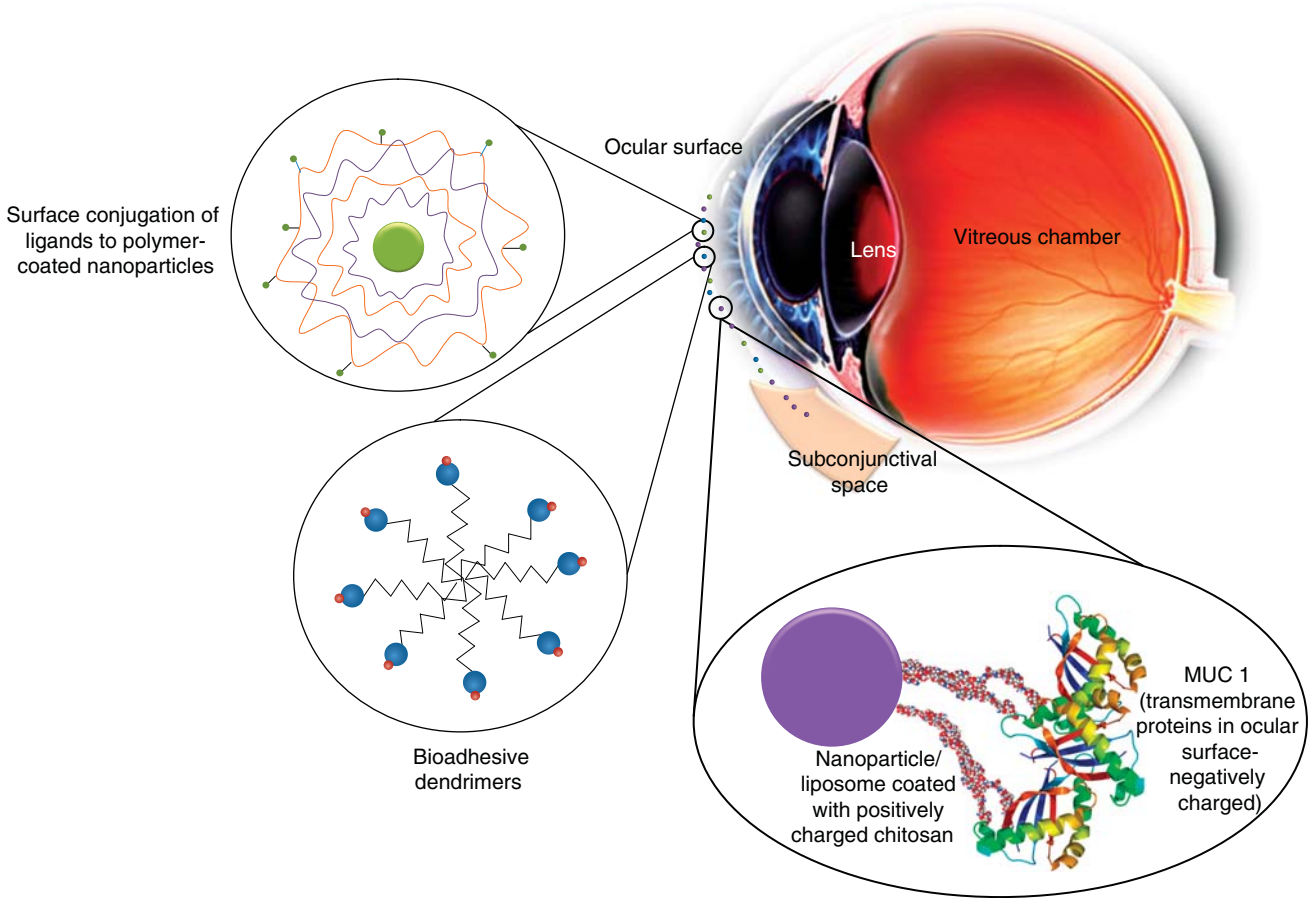

Figure 2. Interaction of positively charged nanoparticles with the ocular surface and subconjuctival space of human eye (inset shows interaction of the chitosan molecules coating the nanoparticle with the negatively charged transmembrane proteins). Chitosan molecules develop molecular attraction forces by electrostatic interactions with the negative charges of mucin, which is determined by the formation of either hydrogen bonds, or ionic interactions between the positively charged amino groups of chitosan and negatively charged sialic acid residues of mucin, depending on environmental pH [6,30].

blinking and demonstrating much greater acceptance compared with viscous Newtonian formulations [51,52]. This rationalizes the efficacy of non-Newtonian vehicles, which include HA and polyacrylic acids, compared with Newtonian formulations containing polyvinyl alcohol or cellulose in a similar viscosity range [44]. Other factors to be considered in the design of an ocular system that will be effectively retained are mucoadhesive and wetting properties [53]. The bioadhesive properties of polyacrylic acid hydrogels and their ability to interpenetrate the mucin at the surface of the eye have been investigated extensively [44,54-57]. Furthermore, Robinson [58] emphasized that the preferred bioadhesive polymers are polyanions such as polyacrylic acids, that is, Carbopol® (Lubrizol, Ohio, USA) 934P and polycarbophil. Acceptability to the pharmaceutical industry must also be considered, for example, Carbopol 934P is lightly crosslinked and has a molecular mass of ~ 3,000,000 Da and is readily soluble in aqueous solutions. Polycarbophil is an anionic bioadhesive water-insoluble crosslinked polyacrylic acid polymer that incorporates large quantities of water on swelling at neutral pH [44,59-61].

Though much mention is made here of these anionic polymers, it is the cationic mucoadhesive polymers that have come to the fore, such as chitosan. Cognisance of the fact that the cornea and conjunctiva possess a negative charge (see **Figure 1**) highlights that mucoadhesive polymers having the propensity to interact intimately with these extraocular structures would increase the concentration and residence time of the associated drug. In this regard, Motwani *et al.* [62] and others have identified the cationic polymer chitosan as a polymer of choice because of its unique properties, including acceptable biodegradability, biocompatibility [63,64] as well as the ability to increase membrane permeability, both *in vitro* and *in vivo* [65], enhance paracellular transport and be degraded by lysozymes in serum. The efficacy of chitosan for ocular drug delivery has been highlighted [66,67]. Its role in bioadhesive nanosystems is elaborated further during the course of this review.

Physiologically, it must be considered that subsequent to topical administration of an ophthalmic drug solution, the following processes become apparent.

- The drug is mixed with the lachrymal fluid. The contact time of drug with ocular tissues is relatively short (1 – 2 min) because of the permanent production of lachrymal fluid (~ 0.5 – 2.2 l/min).
- Approximately half of the drug flows through the upper canaliculus and the other half through the lower canaliculus into the lachrymal sac, which opens into the nasolachrymal duct.
- Drainage of lacrymal fluid during blinking (every 12 s) towards the nasolachrymal duct induces a rapid elimination of conventional dosage forms. The drug is absorbed into the retina-choroid via an extracorneal or sclero-conjunctival route; the iris and ciliary body are presumably supplied via both the transcorneal and the extracorneal pathways.
- Drugs penetrate across the corneal epithelium by means of the transcellular or paracellular pathway. Lipophilic drugs navigate the transcellular route; hydrophilic drugs penetrate primarily by means of the paracellular pathway (passive or altered diffusion through intercellular spaces). The transcorneal penetration appears to be hindered by the binding of the drug to the corneal tissues, after which the cornea may act as a reservoir, slowly releasing drug into the aqueous humor.
- Drugs are distributed from the aqueous humor to the intraocular tissues, that is, iris-ciliary body, lens, vitreous and choroid-retina, and eliminated mainly via aqueous humor turnover and venous blood flow in the anterior uvea.
- Both transconjunctival absorption and transnasal absorption after drainage by means of the nasolachrymal duct are generally undesirable, not only because of the loss of active ingredient into the systemic circulation, but also because of possible side effects [44].

With specific reference to bioadhesive drug delivery devices and enhancing their performance, a five-compartment model was developed [68] that can be used to study the mechanisms involved in transcorneal permeation. The five compartments comprise the tear film, epithelium, stroma, endothelium and aqueous humor, which were assumed to be perfectly mixed and adequately represented by plane sheet barriers of known physical thickness with constant surface area. In this model, four routes of drug loss are evident, namely, lachrymal drainage, conjunctiva absorption, aqueous drainage and iris-ciliary body absorption (**Figure 3**). Through institution of simple mass balances and flux relationships, the investigators converted the compartment model to a series of mathematical expressions.

3. Nanoparticles as bioadhesive ocular delivery systems

3.1 Overview

Nanoparticles for drug delivery are polymerically based and vary in size from 1 to 1000 nm. Varying drug-loading configurations are evident; the drug can be attached to a nanoparticle matrix, dissolved, encapsulated or entrapped, leading to different terminologies, all of which embody the nanosize characteristic – that is, nanospheres or nanocapsules (**Figure 4**) [6,69]. Nanospheres are small solid matricial spheres constituting a dense solid polymeric network, developing over a large specific area into which drug is either incorporated in the matrix of the nanospheres or adsorbed onto the surface of the colloidal carrier; whereas nanocapsules are small capsules formed of a central cavity (oily droplet) surrounded by a polymeric membrane [44]. Ultimately, nanoparticles represent a versatile drug delivery system, having the ability to overcome physiological barriers and guide the drug to specific cells or intracellular compartments by means of passive or ligand-mediated targeting mechanisms [6,19,70-72].

'Although the size of the nanoparticles are in the colloidal range which is more precisely accepted to fall between 1 nm and 0.5 μm for ophthalmic formulations, such a preparation may contain larger particles albeit within the colloidal range stated earlier', stated Nagarwal *et al.* [6]. The dispersion medium may be described as lyophilic and lyophobic. Lyophilic systems are usually easier to prepare and have the greater stability. A dispersion of nanoparticles, being organic molecules, is readily achieved to form colloidal dispersions, and exploitation of this through dispersion in a suitable ophthalmic vehicle would have the advantage of application in liquid form in the same manner as eye drops. The inherent advantages of nanoparticles are intrinsic to their colloidal nature. As ocular drug delivery systems, they are poised to enhance drug bioavailability (especially of poorly water-soluble or poorly permeable drugs) and maintain activity at the site of action, thus enhancing the therapeutic effect (owing to reduced cellular and tissue clearance of drugs, sustained drug delivery, enhanced precorneal residence and uptake of drugs by ocular epithelia). Also, they avoid the discomfort associated with the application of viscous or sticky preparations such as ointments, which cause total blurring of vision on correct administration [6,31]. Compared with normal eye drop formulations, drug-loaded nanoparticles, as a colloidal dispersion, possess the added advantage of enhancing patient compliance, owing to less frequent application and extended duration of retention in the extraocular portion [6,69]. The authors have highlighted that the main problem in ocular therapeutics is to provide and maintain an adequate drug concentration at the site of action, because the poor ocular bioavailability of many drugs implies repetitive instillations. In this regard, nanoparticles have demonstrated promising results over the last couple of decades by being able to enhance the corneal uptake and the intraocular half-lifes of drugs [45,73].

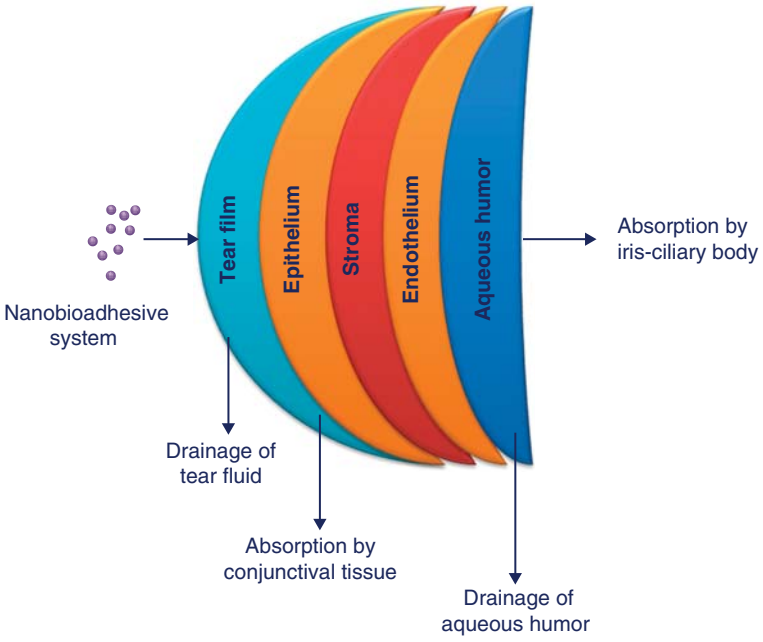

Figure 3. Schematic of the five-compartment model developed for drug delivery devices.
Adapted from [68].

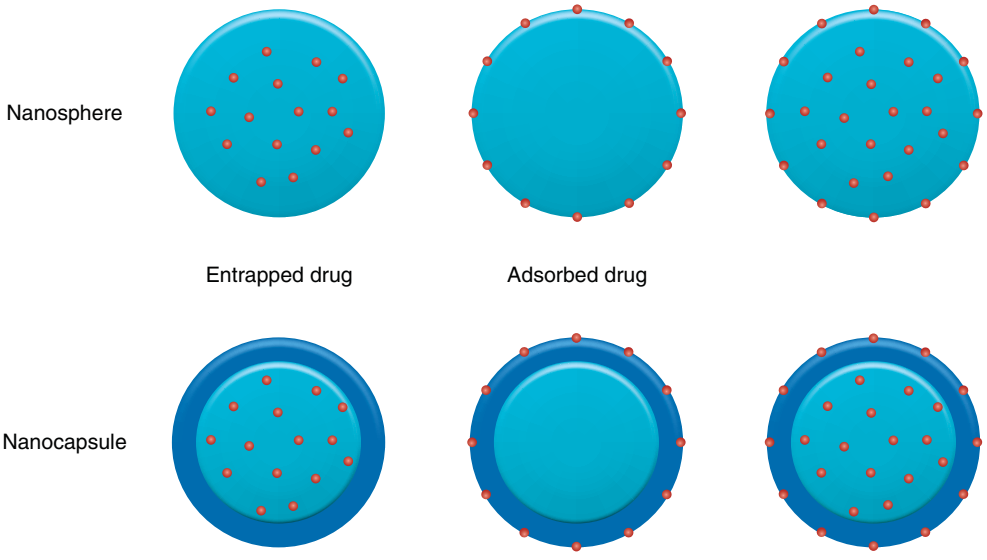

Figure 4. Different configurations of drug-loaded nanoparticles for ocular drug delivery.
Adapted from [6].

Nanoparticles of various sizes functioning as nanobioadhesives for the improvement of ocular drug delivery have been based on polymers and biomaterials such as PLGA, polylactide (PLA), poly-ε-caprolactone, albumin and chitosan [30,31,37,38,74-77]. The stability of colloidal particles in biological fluids containing proteins and enzymes is a crucial issue, because the size of the nanoparticles plays an important role in their ability to interact with mucosal surfaces and, in particular, with the ocular mucosa [6].

3.2 Systematic review of nanoparticulate bioadhesive ocular delivery systems

Over the past few decades, nanoparticles have been the focus of attention of the colloidal systems, and there are numerous

studies reporting on these, not all of which can be discussed extensively in this review, but which warrant mention [78-89].

A study by De Campos *et al.* [10] also highlighted the advantages of colloidal systems for ocular drug delivery in addition to the need to overcome the short residence time of these colloidal systems in the ocular mucosa in the therapy of extraocular diseases, such as keratoconjunctivitis sicca or dry eye disease. Cyclosporin A (CyA) has been identified for its local immunosuppression, which has proved to be effective for the management of the described extraocular disorders. The target sites for the treatment of these diseases are the cornea and conjunctiva, however, the CyA delivery systems previously investigated for delivery of this hydrophobic drug (i.e., oils, emulsions, collagen shields, liposomes and nanocapsules) have been unsuccessful. Oil-based vehicles have been popular but have serious limitations, such as the slow partition rate of CyA into the corneal epithelium [90], the intraocular and/or systemic absorption of CyA [91], and the local side effects associated with the use of oils (symptoms of irritation, blurred vision, itching, transient epithelial keratitis and toxic effects at the corneal level) [92]. These side effects were minimized when CyA-loaded liposomes were used; however, inadequate CyA levels were achieved in the ocular mucosa on administration [93]. Although collagen shields were found to provide a sustained delivery of CyA to the ocular surface, their use is hindered by the ocular irritation and blurring of vision [94]. Calvo *et al.* [27] previously developed CyA-loaded poly-ε-caprolactone nanocapsules in order to improve the ocular penetration of CyA. Although they enhanced the transcorneal transport of CyA while reducing systemic absorption, they failed to provide significant CyA at the ocular mucosa for extended periods of time [27]. The institution of mucoadhesive polymers to improve the drug delivery potential for the management of external ocular diseases was thus considered by De Campos *et al.* [10] for enhancing CyA delivery after Calvo *et al.* [67] demonstrated that introduction of a chitosan coating to the poly-ε-caprolactone nanoparticles dramatically improved the bioavailability of indomethacin in the cornea as well as in the aqueous humor after topical ocular instillation. The potential of the positively charged chitosan for adherence to the negatively charged cornea and conjunctiva has been repetitively highlighted herein for inclusion in colloidal systems to increase the concentration and residence time of the associated drug through its mucoadhesive and absorption enhancer effects. Based on these considerations, the main goals of their study were to associate the hydrophobic peptide CyA to the hydrophilic chitosan nanoparticles for the creation of an improved vehicle for delivery of drugs to the ocular mucosa. A modified ionic gelation technique was used to fabricate the CyA-loaded chitosan nanoparticles, which had a mean size of 293 nm and a zeta potential of + 37 mV. A burst release was observed during the first hour of *in vitro* studies followed by a more gradual drug release over 24 h. Topical instillation of CyA-loaded chitosan nanoparticles in rabbits showed that therapeutic concentrations were achieved in external ocular tissues (i.e., cornea and conjunctiva) for at least 48 h while maintaining negligible CyA levels in inner ocular structures (i.e., iris/ciliary body and aqueous humor), blood and plasma. These levels were significantly higher than those obtained following instillation of a chitosan solution containing CyA and an aqueous CyA suspension. The authors emphasize the applicability of this system at the extraocular level to enhance the therapeutic index of clinically challenging drugs. There was thus favorable and prolonged residence time of chitosan nanoparticles at the ocular mucosa after their topical administration to rabbits [10].

Various other synthetic polymers had, in the meantime, been examined in order to prepare mucoadhesive nanoparticles. Pignatello *et al.* [95] developed nanoparticles composed of Eudragit® RL100 with good ocular tolerance, and no inflammation or discomfort in the rabbit's eye. Positively charged nanoparticles could also be prepared when Eudragit RL100 was combined with PLGA [96].

Hyaluronic acid has been highlighted as a natural, non-irritating polysaccharide showing pseudoplastic behavior with desirable ocular mucoadhesive properties. The study of Barbault-Foucher *et al.* [45] was performed to design a new ocular drug delivery system based on biodegradable nanospheres coated with a bioadhesive polymer. The system was composed of a core of poly-ε-caprolactone surrounded by a corona of the bioadhesive HA molecule. The association of HA with liposomes has been undertaken in previous investigations, using lipid derivatives of HA for incorporation into the liposome bilayers – these had been applied for topical drug delivery in wound healing [97]. Barbault-Foucher *et al.* [45] explored an alternative strategy that included the non-covalent attachment of unmodified HA to the surface of the nanoparticles. This embodied three approaches, namely i) coating the poly-ε-caprolactone core during particle formation by chain entanglement with HA; ii) coating of preformed poly-ε-caprolactone nanosystem by HA adsorption; and iii) coating of poly-ε-caprolactone nanosystem by electrostatic interactions between negatively charged HA and a cationic surfactant used in the formulation (i.e., a cationic lipid, stearylamine, and a preservative usually used in ophthalmic formulation and absorption enhancer, benzalkonium chloride [BKC]). The fabrication of these nanosystems is depicted in **Figure 5**.

The results revealed that HA was strongly attached to nanospheres that had been conferred with a positive charge by cationic surfactant, resulting in intact HA-coated nanospheres. BKC was retained because it was more firmly anchored within the poly-ε-caprolactone matrix and the amount of HA attached was high. In the absence of the surfactant, hydrophilic polysaccharide slightly interacted with the hydrophobic poly-ε-caprolactone nanosystem surface. The group was still in the process of performing bioadhesion studies on this system [45], which have not been reported so far. It must be considered, however, that although various studies in this text have reported on the ocular mucoadhesive properties of

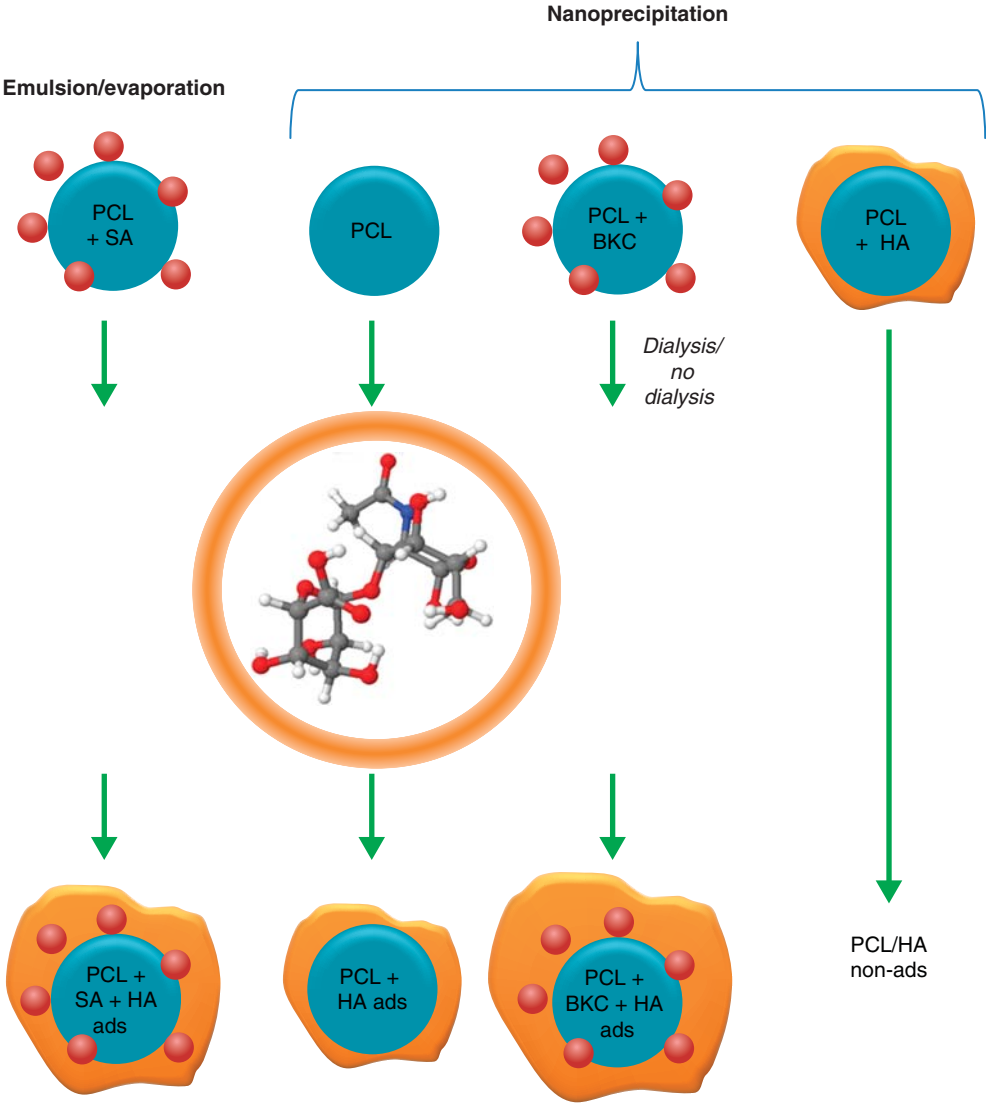

Figure 5. Schematic representation of the different methods used to obtain PCL nanosystems coated with HA.
Adapted from [45].
BKC: Benzalkonium chloride; HA: Hyaluronic acid; PCL: Poly-ε-caprolactone; SA: Stearylamine.

hyaluronic acid, it does not possess permeability-enhancing properties, as chitosan does [73]. Furthermore, the toxicity of stearylamine must be contemplated.

Meanwhile, Vandervoort and Ludwig [17] investigated other natural polymer alternatives for ocular nanoparticle fabrication. They developed and evaluated gelatin nanoparticles loaded with a hydrophilic (pilocarpine HCl) and a hydrophobic (hydrocortisone) drug. Selection of gelatin was based on its biocompatibility and biodegradability. Furthermore, it is derived from collagen that is present in the eye, more specifically in the stroma, and has been used extensively in ocular applications [98,99]. To obtain nanoparticles rather than microparticles, desolvation methods are more appropriate than emulsification or coacervation, and implicate the addition of a desolvating agent (e.g., a salt solution, alcohol, or acetone) to an aqueous gelatin solution. The influence of the gelatin type (A or B) and the pH level during particle preparation was evaluated for pilocarpine-loaded formulations, as these will have important implications on nanoparticle bioadhesivity via corneal interactions. The hydrophobic drug chosen was hydrocortisone. To improve the aqueous solubility of the drug, complexes with cyclodextrins were prepared as a means to optimize the ocular formulations. This facilitated delivery of the complexed lipophilic drug molecule to the membrane surface [100,101]. Also, cyclodextrins can act as penetration enhancers by increasing drug availability at the surface of the corneal and conjunctival membranes, and through the extraction of membrane components, such as cholesterol and triglycerides [102,103]. Slightly negative zeta potential values were measured for all samples. In the case of pilocarpine

HCl-loaded spheres, no influence of the gelatin type or the pH level was observed, which was attributed to the shielding effect of ions present in the dispersion medium. However, a difference in zeta potential value between gelatin type A and gelatin type B particles was measured for hydrocortisone nanoparticles. Compared with the aqueous drug solutions, a sustained release for both drugs was observed. The release kinetics of pilocarpine HCl was close to zero order; however, whether drug concentrations achieved in the cornea and conjunctiva were therapeutically enhanced was not apparent. For hydrocortisone formulations, the release data highlighted a difference in release rate depending on the type of cyclodextrin used [17].

A considerable drawback highlighted by Lemarchand *et al.* [73] for colloidal carriers is their nonspecific interaction with cells and proteins leading to drug accumulation in non-target tissues. Research has thus focused on the development of surface-modified nanoparticles. In this context, PEG was initially identified as an ideal candidate, and was widely used to coat the surface of polyester or PACA nanoparticles [104-106]. The interaction of PEG with the ocular mucosa has thus also been investigated [32]. PEG-coated poly-ε-caprolactone nanocapsules have been demonstrated to accelerate the drug transport across the whole epithelium, compared with chitosan-coated nanocapsules, which favored the retention of the carrier in the superficial layers of the epithelium [73]. If one were to enhance the specificity of the cell-to-cell interaction as specified by Lemerchand *et al.* [73] by means of active targeting, specific ligands would have to be attached to the nanoparticle surface to enable molecular recognition, but because of the absence of reactive groups at the surface of PEGylated carriers, chemical coupling of such ligands is often difficult [107]. Thus oligo- and polysaccharide coatings have been considered as an alternative to the PEG coatings, as well as having the propensity to achieve active targeting as they have specific receptors in certain cells or tissues [108,109]. This is in addition to their well-documented biocompatibilities and biodegradabilities, which are the basic characteristics for polymers used as biomaterials, as well as possessing other activities such as antiviral, antibacterial, antitumoral activities not found in other natural polymers [110,111]. These active targeting capabilities should be exploited further, possibly by attachment of targeting ligands to the oligo- or polysaccharide coating, to achieve different and enhanced modes of bioadhesion (cell-to-cell contact), as elaborated on later in this paper.

Another group focusing on the preparation of ocular colloidal systems from chitosan described a purportedly more simple and effective approach for preparing nanoparticles through the self-assembly of amphiphilic chitosan [112]. No additives were required and the nanoparticles' size could be easily controlled by adjusting the degree of substitution. Also, the hydrophobic microenvironments formed by the association of hydrophobic components enabled the nanoparticles to act as a reservoir of hydrophobic drugs. Chitosan has been modified using poly(ethylene glycol) [113], palmitic acid [114] and deoxycholic acid [115] and used as a carrier for controlled release of diverse agents. However, utilization of self-assembled systems of chitosan as colloidal drug carriers for ocular diseases has received little attention. Yuan *et al.* [112] thus set out to demonstrate the feasibility of amphiphilic chitosan self-aggregated nanoparticles as hydrophobic drug carriers. Cholesterol hydrophobic-modified chitosan (CS-CH) was synthesized, and its self-aggregates were prepared by means of a diafiltration method, using CyA as a model hydrophobic immunosuppressant drug. CS-CH nanoparticles were fabricated by an ethyl-3-(3-dimethylaminopropyl) carbodiimide-mediated coupling reaction. The mean diameters of all samples were < 230 nm. The self-aggregated CS-CH nanoparticles were radiolabeled and their ocular distribution was investigated using single-photon-emission computed tomography and scintillation counter. The CS-CH nanoparticles were retained at the procorneal area, and no radioactivity was detected in the posterior segment. A sustained release of CyA from CS-CH nanoparticles was observed. Institution of this system extraocularly could serve to enhance the therapeutic index of clinically challenging drugs [112]; however, once again the overall bioavailability enhancement was not highlighted – sustained delivery is not the ultimate predictor of an enhanced therapeutic effect. Evidence of uptake into the ocular structures is required.

Following on from their previous work detailing the synthesis of CyA-loaded CS-CH nanoparticles [112], Yuan *et al.* [116] went on to investigate the immunosuppressive effect of rapamycin in chitosan nanoparticles for corneal transplant, including polylactic acid (PLA) in the formulation to improve the hydrophobic drug loading of the nanoparticles. The group thus prepared rapamycin-loaded chitosan/PLA nanoparticles (possessing a size of ~ 300 nm) through a nanoprecipitation method using cholesterol-modified chitosan as a stabilizer and applied these topically to a rabbit allograft cornea as a drop formulation. The immunosuppression was assessed as the median survival time of the corneal allografts treated with nanoparticles, and was ascertained to be 27.2 ± 1.03 days with 50% grafts still surviving by the end of the observation, whereas the group treated with 0.5% rapamycin suspension had a mean survival time of 23.7 ± 3.20 days. The median survival time of the drug-free nanoparticle group and untreated groups were 10.9 ± 1.45 and 10.6 ± 1.26 days, respectively. The results show that a rather enhanced immunosuppression was induced by rapamycin-loaded chitosan/polylactic acid nanoparticles in corneal transplantation [116], indicative of a slight, but not significant, enhancement of bioavailability in the ocular structures.

In addition to negative ocular surface charges, Dillen *et al.* [29] rationalized that most bacteria carry a net negative surface charge, thereby promoting adhesion on positively charged materials [117-119]. Bacterial cell wall polymers such as teichoic acid (Gram-positive bacteria), lipid A (part of the lipopolysaccharide of Gram-negative bacteria), peptidoglycan, and most of the phospholipids are negatively charged [29].

Dillen *et al.* [29] thus examined whether nanoparticles made of PLGA or of a mixture of Eudragit and PLGA would adhere to Gram-positive and Gram-negative microorganisms, in the hope that this adhesion could provide a sustained antimicrobial activity of the incorporated drug against the target organisms implicated in eye infections. Flow cytometric experiments were undertaken to enable visualization of nanoparticle adhesion to microorganisms. In their previous investigations, they demonstrated that nanoparticles consisting of PLGA or a mixture of PLGA and Eudrgit RL 100, prepared via water/oil/water emulsification solvent evaporation, displayed homogenous dispersion of the drug in an amorphous state in the polymer matrix. *In vitro* drug release data fit the Higuchi model [120]. In their later study, Dillen *et al.* [29] used these particles to prepare drug-free and ciprofloxacin HCl-loaded nanoparticles for evaluation of adhesion. The model drug, the fairly water-soluble ciprofloxacin, is one of the most commonly used fluoroquinolones in ophthalmology, owing to its broad *in vivo* activity spectrum against both Gram-positive and Gram-negative ocular pathogens [121]. Adhesion of the cationic Eudragit-containing PLGA nanoparticles to *Pseudomonas aeruginosa* and *Staphylococcus aureus* was thus evaluated. Eudragit-containing nanoparticles possessed a positive zeta potential, whereas PLGA nanoparticles were negatively charged. Following adsorption of Eudragit-containing nanoparticles, an increase in size of *P. aeruginosa* was observed. Flow cytometric analyses confirmed that Eudragit-containing particles possessed stronger interactions with the test organisms than PLGA nanoparticles. Adhesion of particles was more pronounced for *P. aeruginosa* than for *S. aureus*. Cationic Eudragit-containing nanoparticles showed better adhesion to microorganisms than anionic PLGA nanoparticles, purportedly owing to enhanced electrostatic interactions [29]. Previous research showed that the release of the antibiotic from the nanoparticle matrix was prolonged and diffusion controlled. As positively charged nanoparticles interact with bacterial components owing to electrostatic interactions, the continuous diffusion of the antibiotic from the nanosphere matrix through the bacterial membrane can provide a sustained antimicrobial activity [29]. These studies looked at adhesion in a different light. Although prolonged release was reported, the capability of these particles to provide antibiotic concentrations above the minimum inhibitory concentration for common pathogens in the ocular structures should be investigated.

Motwani *et al.* [62] and Gan *et al.* [122] delved extensively into the institution of nanocarriers for ocular drug delivery, exploiting their potential to deliver drugs to the required location, at the appropriate time and at the correct dosage, minimizing drug degradation and metabolism as well as cellular efflux. These systems might be able to enhance drug bioavailability by facilitating transcorneal/transconjunctival penetration [122]. They reiterated that a major challenge for the therapy of extraocular diseases (keratoconjunctivitis sicca or dry eye disease) still exists because of the issue of short residence time of systems in the ocular mucosa [10,62,122]. It is thus imperative to design a mucoadhesive carrier system with improved residence on, and thus enhanced drug delivery potential to, the ocular surface [62]. The use of alginate together with chitosan as a polyionic complex has also been described. Alginates are random, linear and anionic polysaccharides consisting of linear copolymers of α-L-guluronate and β-D-mannuronate residues and are favored for their biodegradable, biocompatible and mucoadhesive nature [123]. Chitosan-alginate (CS-ALG) polyionic complexes are formed through ionotropic gelation via interactions between the carboxyl groups of alginate and the amine groups of chitosan. The complex protects the encapsulant, has biocompatible and biodegradable characteristics, and limits the release of encapsulated materials more effectively than either alginate or chitosan alone [124]. The complex system is also non-toxic, permitting the repeated administration of therapeutic agents. Motwani *et al.* [62] thus developed a slightly modified method to prepare CS-ALG nanoparticles based on the formation of a polyionic complex between the two biopolymers with the aim of optimizing a mucoadhesive nanoparticulate formulation of gatifloxacin for ocular delivery using a Box-Behnken statistical design. Owing to the gentle formulation conditions, this approach may also be suited to the incorporation of gene-based drugs. The designed nanoparticles had an average particle size of 205 – 572 nm and zeta potentials of 17.6 – 47.8 mV. Nanoparticles showed a burst release during the first hour followed by a more gradual drug release during a 24-h period following a non-Fickian diffusion process. The Box-Behnken experimental design achieved optimization of mucoadhesive nanoparticulate carrier systems for prolonged ocular delivery of the antibiotic to the ocular surface [62]. Investigation of the *in vivo* therapeutic efficacy of the system is still necessitated.

4. Bioadhesive liposomes and niosomes

4.1 Overview

Among the progressively innovative ophthalmic drug delivery systems functioning as nanobioadhesives, liposomes seem to be an effective and safe dosage form for ocular therapy [4]. They have not received as much attention as other colloidal carriers, such as nanoparticles, but have been studied for ocular drug delivery by various approaches of administration as a means to improve and facilitate corneal drug transport. Investigations have shown that drug absorption was enhanced when encapsulated in these structures following topical instillation [44].

Liposomes can be defined as biocompatible and biodegradable microscopic vesicles consisting of membrane-like lipid bilayers, composed mostly of phospholipids, surrounding aqueous compartments. These phospholipids are amphiphilic, having a hydrophilic head and a lipophilic tail, which spontaneously arrange in aqueous solution as bilayers (the fatty acid tails, being nonpolar, are located in the membrane's interior,

and the polar heads point outward) to form closed vesicles. Unilamellar lipid vesicles refer to a single bilayer enclosing an aqueous compartment, whereas multilamellar vesicles (MLVs) have more than one bilayer present. According to their size they are known as small unilamellar vesicles (SUVs) (10 – 100 nm) or large unilamellar vesicles (LUVs) (100 – 3000 nm). The surface charge may be positive, negative, or neutral depending on the composition. Lecithin provides liposomes with a neutral surface, stearylamine with a positive charge and phosphatidic acid components with a negative surface charge. The types of liposomal system formulated thus depend on the lipid composition, methods of preparation and the nature of the encapsulated agents [4,125]. Owing to the biphasic nature of liposomes, both lipophilic (bound to the lipid bilayer or 'dissolved' in the lipid phase) and hydrophilic (within the aqueous compartment) bioactives may be incorporated for diverse drug delivery applications. The applicability of liposomes to ocular drug delivery is because of the simplicity of preparation, even though they lack long-term stability in the aqueous form, and their versatility in physical characteristics [44].

Niosomes are non-ionic surfactant vesicles. Like liposomes, niosomes are bilayered structures that can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in the lipid vesicular membrane [126,127].

In the following discussion, it is evident that numerous factors, including lipid composition, surface charge affecting bioadhesion, physicochemical properties of the drug, and the interaction between the drug and the lipid vesicles are necessary in determining whether liposomes can improve ocular delivery. These factors must be considered, and are elaborated on below, with respect to the fate of liposomes in the precorneal site. Lee *et al.* [128] suggested that successful use of liposomes in ophthalmology necessitates that: i) phospholipid vesicles have the ability to overcome the rapid clearance from the precorneal site; ii) liposomes are targeted to the corneal surface; and iii) liposomes are then retained on the corneal surface. This highlights a considerable challenge that has yet to be resolved, but arising from this has been the development of a more in-depth understanding of the fate and interactions of liposomes in the precorneal site [4].

4.2 Chronological review of liposomal bioadhesive ocular delivery systems

Smolin *et al.* [129] was the first study reporting on the utilization of liposomes for ophthalmic therapy. The therapeutic efficacy of idoxuridine in solution and liposomal form in the treatment of acute and chronic herpetic keratitis in the rabbit eye was compared. It was found that the liposome-encapsulated compound was more effective than an equivalent therapeutic regimen of drug solution and herpetic lesions resolved more rapidly in animals treated with the liposomal form of the drug. Thereafter, some early investigations were undertaken [130-139].

Investigations over the years of the potential of liposomes in ocular drug have demonstrated conflicting results with regard to efficacy. Pertinently, liposomes should show enhanced precorneal retention. As an ocular delivery system, liposomal behavior has been partly attributed to surface charge. As with nanoparticles, positively charged liposomes seemed to be preferentially captured/have enhanced binding affinity at the negatively charged corneal surface as compared with neutral or negatively charged liposomes. This interaction is proposed to be electrostatic in nature [132,133]. It is thus anticipated and it has been shown that the degree of association of liposomes with the corneal surface decreases in the order $MLV^+ > SUV^+ > MLV^- > SUV^-$ > MLV,SUV (neutral) [132]. Furthermore MLVs present with a prolonged precorneal retention with regard to SUVs [134]. Also, it is the affinity of liposomes to the conjuctival membrane, rather than the corneal epithelium, that reduces their drainage [133]. Ultimately, the limitation of liposomes is evidenced in their nonspecificity for the cornea. To address these concerns, they have been coated with mucoadhesive polymers, as presented by Davies *et al.* [135].

A positive charge is favored for retention at the ocular surface, but stearylamine, which has generally been used to impart a positive surface charge to liposomes, has been reported to be toxic to cells and appears to be irritating to the eye [138], thus investigators have searched for different substances and means to create vesicles with enhanced retention times or targeting capabilities to the corneal epithelium or to other ocular tissues. Norley and co-workers [140] moved in a new and promising direction and developed immunoliposomes to enhance liposome–corneal interactions. It was found that the attachment of a monoclonal antibody to antiviral agent-loaded liposomes achieved targeting to virus-laden corneal cells *in vitro* and eliminated the infection. This was not the case *in vivo*, however, as the immunoliposomes did not bind to infected cells. Furthermore, experiments with excised cornea indicated that the vesicles had poor penetration into the stroma layer.

Guo *et al.* [141] synthesized and evaluated a series of positively charged phospholipids and cholesterols as membrane components for liposomes. Selected liposome preparations containing these synthetic lipid materials were found to be non-cytotoxic *in vitro* by using a cell growth inhibition assay, whereas liposomes containing more classic positively charged components (stearylamine and cetyltrimethylammonium bromide) showed considerable cytotoxicity. They found that inclusion of the positively charged lipid derivatives into the liposomes significantly enhanced the ocular retention compared with neutral or negatively charged liposomes in an unanesthetized rabbit eye model, owing to molecular association with polyanionic corneal and conjunctival surface mucoglycoproteins. The increased retention was dependent on charge density and rigidity of the lipid bilayer. Liposomes containing cholesteryl esters of amino acids of various carbon chain lengths revealed that the charged amino groups should

extend from the surface of the lipid bilayers for enhanced adhesion and retention. Their investigations thus indicate a specific adhesion of the cationic liposomes to the surface of mucosal tissues [141].

New lipid analogues, benzyldimethylstearyl ammonium chloride and dimethyldioctadecyl ammonium bromide, were used in McCalden and Levy's [142] study to create a positive charge in the formulation of lipid vesicles. The corneal retention time was increased by both lipids compared with an albumin control. The mechanism of adhesion was unclear, but they anticipated increased binding due to the positive charge on the vesicles, liposome-induced impairment of ocular drainage and the formation of loose chemical bonds between the analogues and the cell surface [4]. The drug release capabilities and uptake into the ocular structures of the described liposomal system were not, however, defined.

Once again, the use of mucoadhesive polymers to coat the surface of colloidal carriers and thus retard precorneal drainage enters the discussion [135]. Groups have investigated the possibility of further enhancing the adhesivity of liposomes through addition of a bioadhesive polymer as a means of further promoting the transport of drugs through the cornea and enhancing bioavailability. The effect of including a synthetic mucoadhesive polymer coating was studied by Durrani *et al.* [143], who investigated the effect of Carbopol 1342 on the *in vivo* ocular bioavailability in rabbits of pilocarpine nitrate-loaded liposomes prepared by means of the reverse-phase evaporation method. The enhanced intensity and duration of the miotic response indicated that coated vesicles had an increased ocular bioavailability compared with uncoated vesicles. *In vitro* drug release was more rapid from the uncoated vesicles. However, it appeared that the liposomal form would not offer any advantage over the traditional dosage form as the area under the miotic intensity curve for coated vesicles did not differ significantly from that of a pilocarpine nitrate solution; but an important difference was that the liposomal product displayed a biphasic response characterized by a lower initial intensity but a longer duration of effect. Ultimately, it is still noted that prolonged retention of liposomes and the entrapped substance at the precorneal site can enhance ocular drug delivery. The mechanism for augmented drug absorption is still unclear but it can almost be ruled out that liposomes penetrate the corneal epithelium intact [143].

With regard to encapsulation of immunosuppressive agents, such as the hydrophobic CyA, Milani *et al.* [144] proposed that delivery by means of liposomes could be efficacious in improving the survival rate of corneal grafts in comparison with an oily vehicle, when investigated in a rat model. The ability of liposomes to deliver immunosuppressants was further confirmed by Pleyer *et al.* [145], who substantiated the possibility of using liposomes in a combined delivery system with collagen shields to increase further its slow release property.

Bochot *et al.* [146] aimed to develop a liposomal system incorporating oligonucleotides and dispersed within poloxamer gels in order to prolong the residence time, reduce toxicity, and control the release and limit the degradation of the antisense oligonucleotides following administration into the eye. Antisense oligonucleotides with base sequences complementary to specific target genes offer the possibility of selectively modulating the expression of the gene. They have thus been demonstrated to show inhibitory activity against DNA viruses implicated in ocular diseases such as herpes simplex virus (HSV) in the anterior segment. Their use is unfortunately limited by their poor stability in biological fluids. Encapsulation of the oligonucleotides within liposomes is one approach to be considered. The model oligonucleotide, pdT16, was used, contained within liposomes dispersed within a thermosensitive gel composed of poloxamer 407. The dispersion of liposomes within 27% poloxamer gel was shown to reduce favorably the diffusion of pdT16 from the liposomes, as well as prolong ocular residence. There is no indication, however, of the uptake of these systems into the anterior segment tissues of the eyes.

Li *et al.* [3] noted that efforts are still needed to improve the drug delivery efficiency and to extend the application range of ocular liposomes. They turned their attention to chitosan, but discussed its water insolublity under physiological pH value, which largely constrains its application. Its solubility is dramatically improved by decreasing its molecular mass owing to a decrease in intramolecular hydrogen bonds [147]. They therefore prepared low-molecular-mass chitosan (LMMC)-coated liposomes for ocular drug delivery instituting the poorly water-soluble diclofenac sodium as the model drug, shown to be clinically effective in treating postoperative ocular inflammation and pain after photorefractory keratectomy and cataract surgery [148]. An appropriate mass LMMC was selected (8 kDa) for coating on negatively charged liposome in an attempt to modify the overall delivery mechanism of the liposome and improve its efficiency in ocular drug delivery. The LMMC coating altered the liposome surface charge and slightly increased its particle size. Subsequent to coating, the liposome displayed a prolonged *in vitro* drug release profile, as well as displaying an improved physicochemical stability. To evaluate ocular bioadhesion, *in vivo* precorneal retention was ascertained in the rabbit. LMMC-coated liposomes achieved a significantly prolonged retention compared with non-coated liposomes or the comparative drug solution. As observed for nanoparticles, the coating effected a potential penetration-enhancing effect, facilitating transcorneal delivery of the drug; however, the overall extent of the bioavailability enhancement of the drug following uptake into ocular structures is not evident. Li *et al.* [3] reported no irritation or toxicity following continual administration of the LMMC-coated liposomes. They concluded that the coating significantly enhanced the properties of the liposomes for ocular drug delivery. It was further elaborated that the underlying mechanism of this system and its application in the delivery

of other molecules, such as macromolecule drugs, would be investigated further in continuing studies [3].

The advantages of niosomes over liposomes are seen with regard to their chemical stability, lower cost and availability of materials [114,149]. The combination with bioadhesives such as chitosan propagates an enhancement of these properties to afford good ocular tolerance, improved penetration and enhanced corneal residence time. Kaur *et al.* [127] prepared niosomal vesicles of timolol maleate (TM) by a reverse-phase evaporation method. To increase the retention time of the vesicles and hence TM in the eye, the vesicles were coated with 0.5% w/v chitosan. Their efficacy was compared with a TM solution. Kaur *et al.* [127] reported the pharmacokinetic and pharmacodynamic superiority of the developed ocular formulation of TM, as the intraocular pressure-lowering effect was significantly enhanced. The aqueous humor disposition studies confirmed a significantly higher concentration of TM being achieved with the developed niosomal formulation compared with the solution. The implication is that the niosomal carrier system has improved penetration ability, which indirectly highlights that the systemic availability (through nasolachrymal drainage) would be significantly less, thus reducing side effects.

5. New bioadhesive nanosystems

5.1 Chitosan/cyclodextrin nanoparticles

Earlier in this review, in Section 3.2, the inclusion of cyclodextrins in a nanoparticulate formulation to enhance ocular drug delivery was highlighted [17]. De la Feunte *et al.* [9] highlighted that although chitosan nanoparticles demonstrated success for the association and ocular delivery of lipophilic molecules, certain studies have investigated the prospect of modifying their composition in order to broaden their versatility and minimize their toxicity [150,151]. For example, Maestrelli *et al.* [150] developed a new nanoparticulate drug carrier, combining the benefits of chitosan and cyclodextrins. Cyclodextrins are a family of cyclic oligosaccharides that improve the solubility or stability of hydrophobic molecules through the formation of inclusion complexes [152,153]. The nanocarriers demonstrated adequate potential for the association of hydrophobic molecules (including proteins and genes). In addition, they have shown excellent properties for transmucosal delivery of macromolecules across the nasal mucosal route [154,155]. Their potential for ocular drug delivery, however, has yet to be evaluated. The advantages of each entity could see their combination providing specific benefits as ocular drug delivery systems [9].

5.2 Chitosan-based delivery vehicles for gene therapies

Nucleic acid-based therapies are emerging as an alternative and increasingly significant approach for the treatment of different disorders of the eye [156-160]. Their selectivity offers a distinct advantage over conventional systems. The transfection of the ocular mucosa is of interest for the local treatment of the corneal and/or conjunctival epithelium [161]. However, an obstacle impeding the potential of gene therapy is the delivery of these drugs to the target site and improvement in their retention at the ocular surface [44,162-164]. Numerous synthetic carriers have been proposed recently to achieve this goal [165]. Chitosan-based systems have demonstrated superiority in this regard and the diverse systems implemented to achieve adequate entrapment, retention and transfection are provided in **Table 1**.

5.3 Bioadhesive cubosomes

Gan *et al.* [122], while also searching for a means of improving ocular retention, highlighted that when colloidal systems are dispersed within mucoadhesive gels to enhance adherence to the corneal/conjunctival surface, the high gel viscosity could adversely accelerate the blinking frequency, leading to a feeling of discomfort. They identified monoolein (MO) as a non-toxic, biodegradable and biocompatible material classified as GRAS (generally recognized as safe), and may have distinct pharmaceutical applications in the mesomorphic phase. It may exist in several different phases depending on temperature and hydration. On addition of water at room temperature, the phase sequence is as follows: lamellar crystalline phase (Lc) in coexistence with an L2 phase, lamellar liquid crystalline phase (Lα phase) and the inverted bicontinuous cubic phase (C). Gan *et al.* [122] were most intrigued by the ability of cubic phases to exist in equilibrium with excess water and be dispersed to form cubosomes.

Liquid crystalline phases of MO, such as cubic phases, present interesting properties for a topical delivery system [166-168], including: i) bioadhesivity; ii) permeation enhancement as the structure-forming lipid (MO); and iii) incorporation of compounds independently of their solubility, thus affording protection from physical and enzymatic degradation and allowing sustained delivery [169]. Cubosomes are produced by emulsification of cubic lipid phases in water and are defined as nanoparticulate dispersion systems. Importantly, the dispersed particles have been shown to retain the internal structure and properties of the bulk phase [170-172]. The dispersions hold some advantages compared with the bulk gel, such as larger surface area and high fluidity (low viscosity), and ease of incorporation into other product formulations [173].

Cubosomes are thus seen as innovative drug carriers with attractive capabilities, but research highlighting their potential as ophthalmic drug delivery systems has been scarce [174-176]. Gan *et al.* [122] thus set out to investigate the performance of cubosomes as innovative ocular delivery systems using dexamethasone (DEX), a lipophilic glucocorticoid steroid, as a model drug. It must be noted that continuous application of eye drops of 0.1% dexamethasone for extended periods of time could cause glaucoma accompanied by optic nerve damage, defects in visual acuity and fields of vision, and posterior subcapsular cataract formation and thinning of the cornea or sclera [177]. Thus, an effective ocular delivery system is paramount to achieve a reduction in applications.

Table 1. Design criteria and chitosan-based systems for ocular gene therapy (extracted from de la Fuente *et al.* [9]).

Considerations in the design of an optimal synthetic vector for topical ocular gene delivery		
Gene nanocarriers should be able to entrap the nucleic acids and efficiently deliver them to the ocular cells The carrier must interact with the ocular mucosa and transfect the ocular epithelia under physiological conditions, leading to the desired therapeutic effect		
System	**Description and advantages**	**Challenges**
Chitosan–DNA complexes	CS has been widely explored for its ability to complex nucleic acids and act as an effective gene carrier CS molecular mass was found to affect significantly its ability to transfect mammalian cells. A low molecular mass strongly favors the process of gene transfection	One of the major disadvantages of CS nanocomplexes is related to the simplicity of their structure. Plasmid and polymer are held together by the simple establishment of electrostatic interactions – the presence of polyanions naturally present in the body (e.g., heparin and glycosaminoglycans) may lead to the unpacking and release of the pDNA, before reaching the target site – could explain the lack of information regarding the use of CS complexes for ocular gene delivery The development of more stable nanoparticulate delivery systems – means of improving the potential of CS in ocular gene therapy
Chitosan nanoparticles	These nanocarriers have specific advantages, compared with the simple CS nanocomplexes, e.g., a spherical and homogeneous shape, an improved stability (DNA molecules are not displaced by other anions) and controlled release of the associated DNA and siRNA molecules Further benefit of CS nanoparticles – physicochemical properties, e.g., size and zeta potential – properties that determine the gene transfection efficiency – easily controllable by the adequate selection of the processing parameters	As ocular gene carriers – evidence of their ability to transfect ocular cells *in vitro*; however, *in vivo* data still required Only CS of low molecular mass, i.e., 10 – 12 kDa, was able to cause transfection The capacity of CS nanoparticles to transfect ocular cell lines could be significantly increased by the addition of a second ingredient to the nanoparticle composition, i.e., HA The optimization of CS-based nanoparticles as ocular gene carriers may start by the rational selection of new materials – leads to an increase on the capacity of CS-based nanoparticles to transfect ocular tissues *In vivo* data still required

ALG: Alginate; CS: Chitosan; HA: Hyaluronic acid.

Table 1. Design criteria and chitosan-based systems for ocular gene therapy (extracted from de la Fuente *et al.* [9]) (continued).

System	Description and advantages	Challenges
Chitosan/hyaluronic acid nanoparticles	HA has favorable properties, i.e., mucoadhesiveness, biocompatibility and biodegradability and also ability to interact selectively with the ocular mucosa – HA may act as a targeting ligand to the CD44 receptor expressed in the ocular epithelia Promising behavior of HA/CS nanoparticles as ocular gene carriers and they open up a new potential for the therapeutic application of nucleic acids in ophthalmology	
Chitosan/alginate nanoparticles	CS/ALG nanoparticles have been specifically designed for the prolonged delivery of the antibiotic gatifloxacin to the ocular mucosa Incorporation of ALG is an efficient strategy for increasing the transfection efficiency of CS nanoparticles, as it modulates and improves the delivery of the associated plasmid	Despite the theoretical interest of these nanoparticles for gene delivery to the eye, their application by this particular route remains to be explored
Chitosan–lipid complexes	De la Fuente *et al.* [9] have embraced the idea of generating a new type of nanosystem that combines the positive features of polysaccharides with those described for lipids. The rational mixture of CS with phospholipids or with preformed liposomes has led to the formation of supramolecular hybrid structures Also – their ability to control the release of the entrapped molecules as a function of the lipidic composition In ophthalmology – CS–lipid nanocomplexes can efficiently interact with ocular tissues and enter the ocular cells, but do not compromise the integrity of the cellular membrane The combination should be effective as, on the one hand, CS nanoparticles were able to transfect both corneal and conjunctival cells. On the other hand, liposomes are considered to be efficient systems for transfection – some lipidic compositions have already shown a certain degree of success in ocular gene therapy	Although the efficacy of these nanosystems as gene carriers remains to be proven, the results reported until now suggest their potential as ocular gene delivery systems

ALG: Alginate; CS: Chitosan; HA: Hyaluronic acid.

Gan *et al.* [122] designed new self-assembled liquid crystalline nanoparticles (MO cubosomes) as an ophthalmic delivery system for DEX to improve its preocular retention and ocular bioavailability. DEX cubosome particles were produced by fragmenting a cubic crystalline phase of monoolein and water in the presence of a stabilizer (Poloxamer 407). Small angle X-ray diffraction was undertaken, indicating the diamond cubic phase. The apparent *in vitro* permeability coefficient of DEX administered in cubosomes showed a 3.5 – 4.5-fold increase compared with that of DEX-sodium phosphate eye drops. Preocular retention studies revealed that the retention of cubosomes was significantly longer than that of a DEX solution and Carbopol gel®. *In vivo* pharmacokinetics in aqueous humor was evaluated by microdialysis and indicated a 1.8-fold increase in $AUC_{0\rightarrow 240\ min}$ of DEX administered in cubosomes relative to that of DEX-sodium phosphate eye drops, with an approximate eightfold increase compared with that of DEX suspension. Corneal cross-sections after incubation with DEX cubosomes demonstrated an unaffected corneal structure and tissue integrity, thus emphasizing the good biocompatibility of DEX cubosomes. They concluded that the self-assembled liquid crystalline nanoparticles might represent a promising vehicle for effective ocular drug delivery [122].

5.4 Receptor-mediated bioadhesion

5.4.1 *Luteinizing hormone-releasing hormone agonist and transferrin functionalizations for the enhancement of ocular nanoparticle delivery*

Kompella *et al.* [178] emphasized that even though nanoparticles have been shown to increase drug levels in ocular tissues for some drugs, the percentage uptake of nanoparticles relative to the initial dose is minimal in the anterior segment tissues – a shortcoming noted in several nanobioadhesive systems discussed in this review. Identifying the disadvantages of current nanomedicines, they postulated that nanosystems possessing enhanced mechanisms of uptake by or adhesion to ocular tissues should be developed. To prove this proposition, they assessed the ability of surface conjugation of ligands to increase nanoparticle uptake and transport. In addition to allowing a rapid interaction of nanoparticles with cell surface, they may potentially allow cellular internalization of nanoparticles. Nanoparticles were coated with peptide/protein ligands that enabled recognition of particles by cell surface receptors, namely deslorelin, a nonapeptide luteinizing hormone-releasing hormone (LHRH) agonist, and transferrin, a protein. This investigation was the first time that their uptake characteristics into various layers of bovine cornea and aqueous humor was demonstrated, using an *ex vivo* bovine eye model. In addition, transport studies were conducted with excised cornea and conjunctiva [178]. Ultimately, they found that the uptake of deslorelin- and transferrin-conjugated nanoparticles was several-fold higher compared with plain nanoparticles in corneal epithelium, with the extent decreasing in the presence of free deslorelin or transferrin owing to receptor-mediated endocytosis of ligand-functionalized nanoparticles and competition for the same by free ligands. The rationale for use of transferrin was because the transferrin receptor has been shown to be expressed in human central as well as peripheral corneal epithelium; furthermore, transferrin possesses the ability to enter cells by means of receptor-mediated endocytosis and subsequent transcytosis. This group also demonstrated that deslorelin, an LHRH agonist, can be internalized and transcytosed across cell monolayers and epithelial tissues and that LHRH receptors are expressed on bovine corneal epithelium as well as the conjunctiva. Such approaches show promise, but *in vivo* studies are still required.

5.4.2 *Lectins for receptor-mediated bioadhesion*

The use of lectins is another approach that exploits receptor-mediated bioadhesion [179]. The corneal and conjunctival epithelia of humans and other species possess lectin-binding sites. Lectins are proteins that recognize and bind to sugar complexes attached with high specificity to proteins and lipids. Conjugation to lectins that adhere to the corneal and conjunctival epithelia could purportedly result in prolongation of drug delivery to the eye. This true effectivness of this approach has yet to be investigated extensively *in vitro* and *in vivo*.

5.5 Nanosized new dendrimers for ocular drug delivery

The emergence of dendrimers as new drug delivery systems has been exploited recently. [180]. Dendrimers, defined as macromolecules with repeated branching characterized by their orderly and symmetrical structure, possess physical properties that are dictated by the functional groups on the molecular surface, which can be synthesized with anionic carboxyl, cationic amine, or non-ionic hydroxyl groups [181]. Consideration of their applicability to ocular drug delivery is because of their hydrophobicity and bioadhesive properties. However, in addition to understanding their ocular retention and drug release characteristics, the ocular toxicity of dendrimers needs to be investigated thoroughly in future studies, as well as their long-term stability and compatibility with drugs [181].

Durairaj *et al.* [182] investigated the potential of dendrimeric polyguanidilyated translocators (DPTs), which are nanosized new dendrimers that efficiently translocate molecules across biological barriers. These structures were therefore applied as an ophthalmic delivery vehicle for gatifloxacin (possessing good aqueous solubility), and their *in vitro* and *in vivo* delivery potential was established after topical application. The gatifloxacin (GFX) solubility-enhancing property of a six-guanidine group-containing dendrimer (g6 DPT) was investigated as a function of pH and dendrimer concentration. The dendrimer formed isotonically stable, nanosized (346 nm) complexes with GFX by means of ionic bond, hydrogen bond and hydrophobic interactions. The dendrimer gained rapid entry

into the human corneal epithelial cells (within 5 min) and increased the transport of the aqueous-soluble GFX by 40% across bovine sclera-choroid-retinal pigment epithelium in 6 h. DPT-GFX showed a three times faster killing rate for MRSA when compared with GFX alone. *In vivo* administration of DPT-GFX resulted in ~ 13-fold and ~ 2-fold higher AUCs for tissue concentrations in conjunctiva and cornea, respectively, when compared with GFX after a single dose; and a single dose of DPT-GFX sustained aqueous humor and vitreous humor drug levels during the 24-h study [182].

6. Conclusion

Numerous bioadhesive ocular nanosystems have been developed over the decades, as seen from this review. Most systems exploit the electrostatic interaction between the surface of the colloidal particle – which may or may not be coated with a favorable mucoadhesive polymer, such as chitosan – and the negatively charged ocular surface. For example, as seen for ocular liposomal systems, from biodisposition and pharmacological studies, it can be ascertained that vesicles carrying a positive surface charge often outperform vesicles with a neutral or negative charge. In the case of nanoparticles, chitosan has been exploited extensively as a mucoadhesive coating, whereas new gene therapies see it as the main carrier component, in combination with other mucoadhesive, penetration-enhancing, or lipophilic components. Receptor-mediated bioadhesion and the institution of dendrimers may be seen as emerging approaches that beg further investigation.

7. Expert opinion

7.1 Toxicity considerations

In noting the advantages of new developments in nanobioadhesives for enhancing topical ocular delivery, the other side of the coin must also be considered. It comes as no surprise that the same properties that make nanosystems attractive for biomedical applications may confer reactivity in biological systems and lead to toxicity. For topically administered nanosystems, aggregation and tissue accumulation must be considered. Nanosystem aggregation may block cell metabolism and could impair tissue function, for example, blockage of the lachrymal drainage punctum and impaired tear film recycling can occur owing to aggregation of topically applied nanosystems on the ocular surface. Furthermore, distortion of the ocular tissue architecture leading to altered function could result from indiscriminate nanosystem accumulation in ocular tissues [183]. A very important consideration with nanosystems is toxic effects that may occur as a result of the actual approaches that are instituted to enhance ocular drug bioavailability, which is the presence of high levels of the loaded drug in a non-target tissue – the reason why effective targeting strategies should be considered as bioadhesive mechanisms.

7.2 Modeling for prediction of the nanobioadhesive potential of ocular delivery systems

As nanobioadhesive systems become more intelligent, the need for new and enhanced methods of prediction or assessment of their bioadhesive capabilities, permeation of the ocular surface, and bioavailability enhancement potential becomes paramount. As discussed, animal models for the evaluation of ocular delivery systems do not always translate well to the behavior of the system in the human eye. Simulation is the imitation of a real thing, state of affairs, or process. The act of simulating something generally entails representing certain key characteristics or behaviors of a selected physical or abstract system. In drug delivery system design, simulation is used extensively for scientific modeling to gain insight into system functioning. A computer simulation is an attempt to model a real-life or hypothetical situation on a computer so that it can be studied to see how the system works. By changing variables, predictions may be made about the behavior of the system. Computer simulation has become a useful part of modeling for us in the pharmaceutical sciences for prediction of delivery system behavior and delivery system optimization. Investigators have developed a simulation model of the human eye, which was set up based on information obtained from cadaver eyes, using a specific software program for a supercomputer. This program was used specifically to determine the physical and mechanical conditions of striking foreign bodies causing intraocular foreign body injuries [184].

Several computer-based simulation modeling/programs are now available. These programs may use: computational methods, including molecular mechanics, molecular dynamics, and semiempirical and *ab initio* molecular orbital methods (e.g., HyperChem®); modeling, simulation, visualization, development, documentation and deployment (Mathematica®); or modeling of complex relationships between inputs and outputs or to find patterns in data (artificial neural networks programs). In the design of nanobioadhesives, investigators should be aiming for systems that are not only well retained on the ocular surface but also taken up into the ocular structures. The simulations generated by these programs may be increasingly useful for deriving intensive models of bioadhesion, permeation/penetration of ocular structures, and drug release kinetics where *in vitro* models (e.g., textural analysis) fall short and appropriate *in vivo* animal models are not available. Such simulations could prove increasingly useful, especially with the new generation of targetable systems.

7.3 Future developments

Efficacy has been demonstrated in many of the nanosystems; however, in some instances, no dramatic improvement is pinpointed in the bioavailability at the corneal/conjunctival surface compared with conventional systems, specifically when a mucoadhesive coating is not included. In some instances, no quantitative description of the actual improvement in drug bioavailability to the ocular structures is offered (knowing that the ocular bioavailability of a common aqueous

formulation is < 10% [185]). The actual permeation/uptake of the nanobioadhesive systems into the ocular structures is often not demonstrated. Furthermore, *in vivo* studies are generally reported in an animal model and the discrepancy in anatomy and physiology with the human eye highlights that results cannot be directly translated. In terms of the design of nanobioadhesive systems for ocular drug delivery, pharmaceutical scientists need to take a fresh look in another direction, to identify where future opportunities lie.

To provide a glimpse of what is possible, mucins (which have been exploited as a simple mucoadhesive coating) could be attached to the surface of the nanosystem to attain enhanced adhesion to ocular mucins for the creation of smart cell-adhesive nanocarriers. As an example, Johnson *et al.* [186] prepared microtubes functionalized with the highly glycosylated protein mucin. As mucin is one of the major components of mucoadhesion, such structures, if further nanosized, may aid in both improving the localization of drug delivery and delivering molecules that were previously problematic by other delivery methods. Alternatively, as a more progressive approach, anti-mucin monoclonal antibodies could be conjugated to the surface of the colloidal carrier to achieve active targeting (as attempted, but not successfully achieved, under Section 4.2 by Norley *et al.* [140] through the design of immunoliposomes following attachment of monoclonal antibody). The potential for this approach to targeting has been highlighted for certain cancers, where nanoparticles could potentially be targeted to mucin that is, for example, expressed on the surface of ovarian cancer cells (MUC1, MUC4 and MUC16). The proposed mucin-targeted ocular delivery system could not only enable specificity, but also increase the residence time of the nanosystem at the ocular surface. The further exploration of alternative nanobioadhesive systems, such as cubosomes, is also promising.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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