EXPERT OPINION

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Ocular application of chitosan

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Introduction: A major problem in ocular therapeutics with classical formulations is the maintenance of an effective drug concentration at the site of action for a long period of time. Enhancement of ocular bioavailability with increased dose penetration and longer retention time at desired sites can be achieved by recent formulations. Chitosan stands out with its unique structural advantageous characteristics for different types of formulations like *in situ* gelling systems, micro- and nanoparticles, inserts, etc.
Areas covered: In this review, the authors focus on ocular therapeutics and the characteristics that make chitosan more acceptable in ocular applications.
Expert opinion: Chitosan seems to be one of the most promising polymeric carriers for both hydrophilic and lipophilic drugs for ocular application.

Keywords: bioavailability, chitosan, ocular drug delivery, safety of chitosan

Expert Opin. Drug Deliv. (2012) 9(6):701-712

1. Introduction

Ocular drug delivery remains as one of the most challenging tasks for pharmaceutical scientists with the unique pharmacodynamic and pharmacokinetic properties of eyes [1,2]. The unique structure of the eye restricts the entry of drug molecules to the required site of action. Therefore, the major problem 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 [3,4].

Current ocular therapeutic options are unfortunately limited due to the low systemic access owing to the blood–retinal, blood–aqueous and blood–vitreous barriers. Oral therapy for ocular diseases requires high doses of active agent to reach therapeutic concentrations at the site of action which may cause severe side effects [1,5]. The most common and well-accepted route is the topical administration having two different purposes, to treat superficial diseases such as infections (e.g., conjunctivitis, blepharitis, keratitis sicca) and to provide intraocular treatment through the cornea for diseases such as glaucoma or uveitis [3,6-8]. Following topical instillation of an eye drop, drug is subject to a number of very efficient elimination mechanisms such as tear drainage, binding to proteins, normal tear turnover, induced tear production and non-productive absorption via the conjunctiva. Typically, drug absorption is virtually complete in 90 s due to the rapid removal of drug from the precorneal area. Additionally, cornea is poorly permeable to both hydrophilic and hydrophobic compounds resulting in approximately 10% or less absorption of the topically applied dose into the anterior segment of the eye [9-12].

Classical attempts for improving the ocular bioavailability of drugs mostly include the use of viscosity enhancers (e.g., cellulose derivatives), mucoadhesive polymers (e.g., polysaccharides) and *in situ* gel-forming systems [4]. As a consequence, many strategies were developed to enhance the bioavailability of drugs instead of the standard treatment consisting of frequent instillations which can create incompliance, toxic side effects and cellular damage at the ocular surface [12-14]. Among these approaches, two main strategies are to increase the corneal permeability and to prolong the contact time with the ocular surface [7,15].

10.1517/17425247.2012.681775 ISSN 1742-5247

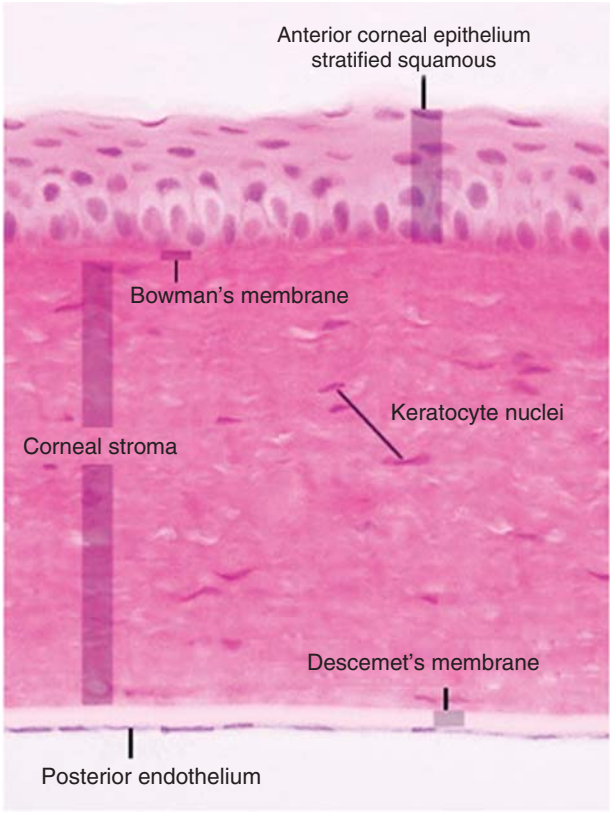

Figure 1. Layers of cornea.
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Penetration enhancers were used in many studies in order to enhance the corneal penetration [4,6,16,17]. However, these enhancers generally exhibit their effects by inducing morphological changes in the corneal membrane and occasionally lead to adverse effects such as irritation in large doses. Therefore, the amount of penetration enhancers needs to be minimized to prevent undesirable side effects [6,9].

A further approach to optimize the efficacy of ocular dosage form is the implementation of the mucoadhesive concept which is successful in buccal and oral applications [13,18]. Ocular bioavailability from a mucoadhesive dosage form depends on the polymer's bioadhesion properties which are affected by its swelling, hydration time, molecular weight and degree of crosslinking. Other factors such as pH, mucin turnover and disease state also affect bioadhesion [19].

'Bioadhesion' can be described in simple terms as the attachment of a synthetic or biological macromolecule to a biological tissue. An adhesive bond may form either with the epithelial cell layer, the continuous mucus layer or a combination of the two. The term 'mucoadhesion' is used specifically when the bond involves mucous coating and an adhesive polymeric device while 'cytoadhesion' is the cell-specific bioadhesion. The mechanism of bioadhesion between mucin and mucoadhesive polymer is usually analyzed based on the molecular attraction and repulsion forces and depends on electronic, adsorption, wetting, diffusion or fracture theories [20]. Due to the attraction theories mentioned above, most cationic macromolecules can interact with the anionic components of superficial cellular glycoproteins. Moreover, the interior of tight junctions (pores) is highly hydrated and contains constant negative charges. A change in the relative concentration of specific ion species in the pores causes alterations in tight junction resistance leading to loosening or opening of the pores [12]. The mucus layer which is secreted onto the eye surface by the goblet cells is associated intimately with the glycocalyx of the corneal/conjunctival epithelial cells [13]. To prolong the residence time of drugs in the preocular area, bioadhesive drug delivery systems were developed taking advantage of the presence of a mucin–glycocalyx domain in the external portion of the eye [20].

Mucin is negatively charged at physiological pH (pH 7.4) owing to the presence of sialic acid groups at the terminal ends of the mucopolysaccharide chain resulting in the preferential uptake of cationic drug carriers [19]. Therefore, use of positively charged formulations is the most common approach to enhance the bioavailability of the topically applied ocular formulations. Development of molecular attraction between the negatively charged corneal and conjunctival surfaces with the positively charged formulations by electrostatic interactions increases the bioavailability at the ocular target site [21-24].

Most common approach for designing a cationic formulation is the addition of a cationic agent such as stearylamine (octadecylamine). This may lead to irritation and/or toxic effect and therefore more precaution is required at the configuration step of the formulations [1,10,22,25]. Polymers with self-cationic character can be used instead of adding cationic agents [26-28]. The intimate contact ability of cationic mucoadhesive polymeric systems will undoubtedly improve ocular bioavailability by high drug concentration at the absorbing corneal area resulting in high drug flux through the absorbing tissue [4,29]. Prolonged contact time may also increase the local permeability of high molecular weight drugs [9].

Chitosan with its hydrophilic and cationic character is investigated widely for its potential as an excipient in oral and other pharmaceutical formulations. This polysaccharide is being studied for topical application for ophthalmic [4,9] and also cosmetic purposes [30,31]. Chitosan has attracted a lot of attention in ocular applications as a potential penetration enhancer across the mucosal epithelia due to its polycationic, biocompatible and biodegradable nature together with its mucoadhesive property [3,4,21,31,32]. Chitosan is generally regarded as a non-toxic and non-irritant material and the properties attributed to chitosan make it an excellent candidate for ocular application [30].

2. Ocular drug delivery

The front part of the eye globe is clear and colorless and is called the cornea. It contains no blood vessels but is rich in

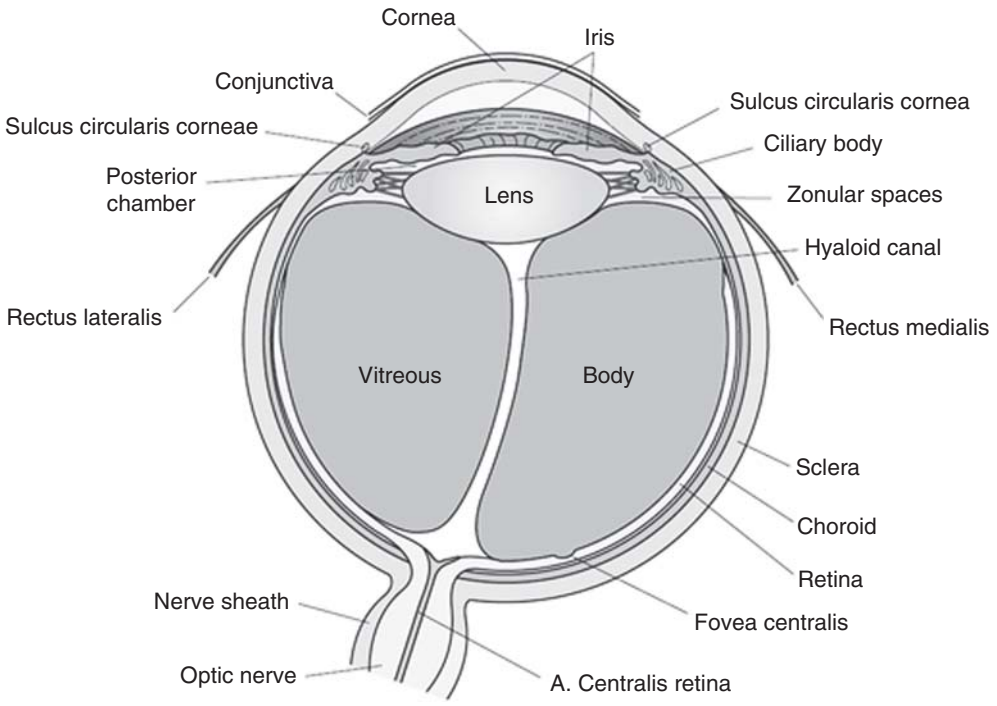

Figure 2. Anatomy of the eye.
Adapted with permission from RPS Publishing [36].

nerve endings. Cornea consists of three major layers: outer epithelium, middle stroma and inner endothelium (**Figure 1**). When drug products are applied topically to the eye, they first encounter the cornea and conjunctiva which represent the primary barriers to drug penetration [33]. Drugs penetrate across the corneal epithelium via the transcellular pathway (mainly for lipophilic drugs) or the paracellular pathway (for hydrophilic drugs). Transcorneal penetration seems to be hindered by the binding of drug to the corneal tissues which are claimed to act as drug reservoirs [3]. The epithelium and endothelium of the cornea are rich in lipid content making them barriers to the permeation of polar, hydrosoluble compounds. The hydrophilic layer stroma contains 70 – 80% water and presents a barrier to the permeation of non-polar, liposoluble compounds [33]. Stroma, containing structural and cellular elements including nerves, lymphatics and blood vessels, is attached loosely to the underlying sclera [34,35].

Cornea shows permselectivity also. It has an isoelectric point (pI) of 3.2. At pH values above the pI it carries a negative charge and is selective for positively charged molecules. On the contrary, it carries a net positive charge at pH values below the pI. Conclusively, a positively charged molecule passes through the cornea more effectively at physiological pH (7.4) than a negatively charged molecule [9].

Conjunctiva is a thin transparent mucous epithelial barrier which lines inside the eyelids and covers the anterior one-third of the eyeball (18 cm^2) [34,36]. The respective portions of conjunctiva are referred to as the palpebral and bulbar conjunctiva. Area joining palpebral and bulbar conjunctiva is called the fornix (*forniceal conjunctiva*). Conjunctiva is composed of two layers, the outermost epithelium and the underlying stroma (*substantia propria*). Epithelium is covered with microvilli and consists of stratified epithelial cells of 5 – 15 layers. Epithelial cells at the apical side connect with each other by tight junctions which play a barrier role in permeability [34]. Several polymers were identified that can safely and reversibly disrupt cellular tight junctions. Among them chitosan appears to be a very promising candidate. *In vitro* and *in vivo* studies demonstrate chitosan's ability to increase passive diffusion of compounds across biological membranes probably due to its effect on tight junction proteins [37].

Pharmaceutical scientists show continuing interest in ocular drug delivery due to challenges in unique pharmacodynamic and pharmacokinetic properties of the eye (**Figure 2**) [1,2]. Selection and design of the route of administration of drugs take into account the quantity of drug present at the site of action to produce the desired action. Certain portions of the eye are more accessible to drugs given by one route than drugs given by another route [38]. For example, corneal route was shown to be the predominant pathway for more lipophilic drugs for delivery to iris while less lipophilic drugs need conjunctival/scleral penetration for delivery into the ciliary body [39].

Drugs also vary in their ability to cross capillary and mucous membrane barriers [38]. Common routes of administration for anterior-segment drug delivery are topical instillation and

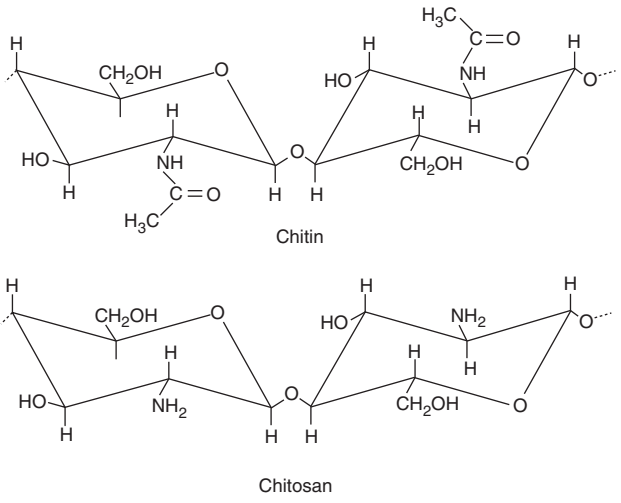

Figure 3. Structure of chitin and chitosan.
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subconjunctival injection while common routes for posterior-segment drug delivery include periocular and intravitreal injections, systemic dosing and topical application [3,38].

Direct injection can either be periocular (subconjunctival or retrobulbar) or intravitreal. This type of application has very low patient compliance. Therefore, direct injection is used when relatively large doses of drug are required at the site of action for immediate efficacy such as antibiotics and local anesthetics [38].

When a drug is administered through the systemic route, some portions of the eyes show no bioavailability because those portions are not vascularized (e.g., cornea) [38]. The second drawback of the systemic application is the exposure of the whole organs to high doses of drug to maintain therapeutic concentrations at the site of action which may result in severe side effects [1,5]. However, systemic drug administration may be necessary for some instances to back up other treatments like the use of diuretics for treating glaucoma and as a supplementary to topical antibiotic application [38].

Topical route is the most common route of administration of drugs targeting both segments of the eye [3,38]. Topically applied ocular agents produce effective levels mainly in the anterior segment [38]. There is high patient compliance over 90% for topical administration because of the ease in use and no requirement for a qualified person for application (compared with ocular injections) [3,39].

3. Chitosan: why we prefer?

3.1 Source, structure and physicochemical properties of chitosan

Chitosan is denominated to deacetylated chitins in various stages of deacetylation and depolymerization and therefore not easily defined in terms of its exact chemical composition (**Figure 3**) [30,40].

Chitin is present in the exoskeletons of crustaceans, cuticles of insects and cell walls of most fungi [41]. However, applications of chitin are limited when compared with chitosan since chitin is structurally similar to cellulose, but chemically inert. Acetamide group of chitin can be converted into an amino group to give chitosan by treating chitin with a concentrated alkali solution [42].

Chitosan is a heteropolymer containing both glucosamine and acetylglucosamine units [41,43]. Chitosan is one of the most promising polymers for drug delivery through the mucosal routes because of its polycationic, biocompatible and biodegradable nature as well as its mucoadhesive and permeation-enhancing properties [3,21,31,32].

Presence of amine group explains its unique properties among other biopolymers, its cationic behavior in acidic solutions and its affinity for metal ions. Cationic metal sorption can occur either through chelation mechanisms in nearly neutral solutions or through electrostatic attraction; ion exchange for metal anions happens to occur in acidic solutions [41].

A clear nomenclature with respect to different degrees of N-deacetylation between chitin and chitosan was not defined. Chitosan is not considered as one chemical entity but varies in composition depending on the manufacturer [30].

Commercially available chitosan is either in free base form or in different salt forms. Hydrochloride, glutamate and lactate salts being the most common, they appear as solutions, dry flakes and fine powders which are odorless, white or creamy-white, which vary in molecular weight in a wide range and vary also in degree of deacetylation and viscosity [30,44,45].

Better mucoadhesion was observed for chitosan having higher molecular weight (approximately 1400 kDa) compared with lower molecular weight chitosans (500 – 800 kDa). However, glutamate salt of a relatively low molecular weight chitosan (25 – 50 kDa) also exhibited good mucoadhesion [46].

Glass transition temperature of chitosan is 203°C [13] and chitosan degrades before melting which is a typical behavior for polysaccharides with extensive hydrogen bonding. This property makes it necessary to dissolve chitin and chitosan in appropriate solvent systems to impart functionality [45]. However, fiber formation is quite common during precipitation and chitosan may look 'cottonlike'. Aqueous solution (1% w/v) with the density of 1.35 – 1.40 g/cm^3 has a pH value of 4.0 – 6.0 [30]. Chitosan exhibits both pseudoplastic and viscoelastic rheological behaviors [13].

Chitosan does not cause allergic reactions or rejection due to its biocompatible character. It breaks down slowly to harmless amino sugars which can be absorbed completely by the human body [42].

Various sterilization methods such as ionizing radiation, heat, steam and chemical methods can be suitably adopted for sterilization of chitosan in clinical applications [42].

3.2 Ocular application of chitosan systems

Chitosan is proposed as a material for potential ocular drug delivery by reports claiming its biodegradability, biocompatibility and good stability [47].

Approaches to penetration enhancement of topically applied drugs considered chitosan as a superior mucoadhesive cationic polymer due to its ability to develop molecular attraction forces with the negative charges of mucin by electrostatic interactions. Interactions are 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 the environmental pH [13,48-50].

Various chitosan derivatives were synthesized not only to improve mucoadhesion but also to enhance penetration of drugs and peptides through the mucosa by opening the tight junctions between epithelial cells or by intracellular routes. However, *in vitro* studies showed that cell surface binding and absorption-enhancing effects were reduced in cell lines covered with mucus. Quaternized *N*-trimethyl chitosan and *N*-carboxymethyl chitosan were proved to be potent intestinal penetration enhancers and therefore it was thought that they could be of interest in ocular formulations when high aqueous humor levels are required [13].

Chitosan formulation needs no addition of organic solvent during preparation since chitosan is soluble in weak acid solutions. Chitosan binds strongly to negatively charged cellular and mucosal surfaces resulting in the improvement of drug bioavailability and thus reduces the administration frequency which are all advantageous for controlled delivery [51].

Many researchers studied chitosan formulations in the form of several delivery systems like solutions, gels, liposomes, emulsions, nanoemulsions, nanostructured lipid carriers (NLC), micro- and nanoparticles, inserts and chitosan conjugates aiming the enhancement in the bioavailability of active agents [3,4,13].

Felt *et al.* [14] studied the precorneal retention of tobramycin in chitosan solutions and they found that even chitosan concentration as low as 0.5% is sufficient to ensure a significant enhancement in the residence time of ophthalmic solutions.

Yuan *et al.* [11] demonstrated the feasibility of amphiphilic chitosan self-aggregated nanoparticles as hydrophobic drug carriers for ocular application with elevated retention ability at the procorneal area and no radioactivity in the posterior segment with a sustained release of entrapped drug over 48 h.

De Campos *et al.* [21] showed the advantages of cationic systems in ocular drug delivery owing to their close contact with the corneal and conjunctival surfaces. In this study, increase in delivery to external ocular tissues without compromising inner ocular structures and providing long-term drug levels in target layers could be maintained.

Antibacterial activity of chitosan itself is also an advantage in ocular application because secondary infections due to the diminished tear secretion containing antibacterial lysozyme and lactoferrin are frequently observed especially in *kerato conjunctivitis sicca* [13].

It was reported that a radiolabeled chitosan formulation remained at a constant viscosity at the ocular surface five times longer than other polymeric solutions [13].

Among the mucoadhesive polymers investigated, the cationic polymer chitosan has attracted a great deal of attention because of its unique properties [21].

3.3 Polymer safety

Chitosan was designated as Generally Recognized As Safe (GRAS) material in the USA not only as a pharmaceutical excipient but also for use in foods. It was also listed as a food additive in Japan, Italy and Finland [43,52,53].

Chitosan has an industrial use as a flocculant and chelating agent in the clarification of beverages and as a fungicide for crop protection [43].

Besides its food supplementary role, chitosan is being investigated widely for use in pharmaceutical formulations owing to its properties such as biodegradability, low toxicity and good biocompatibility [30,32,42,44,54].

Levels of 4.5 – 6.75 g chitosan taken daily by human volunteers were shown to have no adverse effects. No clinically significant symptoms including allergic response were found in short-term human clinical trials of up to 12 weeks. However, a low incidence of mild and transitory nausea and constipation was reported [43].

Chitosan degrades under the action of ferments. Degradation products are non-toxic and easily removable from the organism without causing concurrent side reactions. Chitosan possesses an antimicrobial property and absorbs toxic metals like mercury, cadmium, lead, etc. In addition, it has a good adhesion and coagulation ability and also an immunostimulating activity [42].

Toxicity of chitosan was discussed in detail by Baldrick [43]. He concluded that the 50% lethal dose (LD_{50}) of 16 g/kg (for rats 30 g/kg) body weight obtained in mice was close to sugar or salt and therefore chitosan can be classified as safe for pharmaceutical applications [42-44].

Toxicities of chitosan and its derivatives evaluated by 50% cellular growth inhibition (IC_{50}) values against MCF7 and COS7 cells were presented in the literature [55]. The resulting finding was that most chitosans and their derivatives were not significantly toxic compared with a toxic polymer such as polyethylenimine (LD_{50} < 20 μg/ml) [54].

Yoksan and Chirachanchai [56] stated that LD_{50} of amphiphilic chitosan nanospheres in rats are higher than 2 g/kg body weight implying that the nanospheres are non-toxic. However, Omara *et al.* [57] reported alterations in liver enzymes even at low doses of oral chitosan treatment in both male and female mice. Chitosan-induced histopathological changes (hypercellularity of glomerulus and degeneration in renal tubules with interstitial hemorrhage) were detected in livers and kidneys of rats. Increase in severity was found to be dose-dependent. Urea was significantly elevated mainly due to hepatic failure caused by metabolic interruption in protein metabolism which converts amino acids and ammonia to urea [57].

The cytotoxicity and mucoadhesion behaviors of chitosan nanoparticles were analyzed using lactate dehydrogenase and

MTS cell proliferation tests which are utilized to evaluate the effect of nanoparticles on both cellular viability and membrane integrity [29]. Analysis results by Student's t-test demonstrated no statistical difference between control media and nanoparticles with different sizes for the two tests. This result highlighted that chitosan nanoparticles are not harmful to the cells and the size has no effect on cytotoxicity [29].

In parallel to researchers reporting no apparent toxic effects observed for chitosan nanoparticles at low concentrations, death and malformation of zebrafish embryo occurred with increasing chitosan nanoparticle concentration. Almost 100% of mortality was observed at a concentration of 40 mg/l for 200 nm chitosan nanoparticles. Therefore, toxicity was decided to be dose-dependent and considered at high concentrations. It was also found that small chitosan particles showed high toxicity in the zebrafish embryo model identifying that toxicity of chitosan nanoparticles was size-dependent [47].

Loh *et al.* [58] studied the uptake and cytotoxicity of chitosan nanoparticles in human liver cells and they concluded that chitosan nanoparticles were less cytoadhesive than the chitosan molecule itself. However, nanoparticles were rapidly internalized by bi-potential human liver cells (BHAL), the human liver cell line derived from non-tumorous tissues. Internalized nanoparticles were found to lead to a reduction in cell viability and proliferation while the extracellularly associated chitosan molecules appeared to promote cell proliferation. Hence drug delivery strategies using chitosan nanoparticles as a vehicle need to consider their adverse effect on cells which are often induced to proliferate in chronic liver disease [58].

There are many examples of studies reporting the decrease in chitosan toxicity following polyethylene glycolation (PEGylation) [45,59]. PEGylated chitosan was found to be non-toxic against cancer cells by Casettari *et al.* [45] and they identified the PEGylated chitosan copolymer as a promising candidate compound with potential use in a wide range of biomedical applications. IC_{50} of mitomycin-C decreased from 1.97 ± 0.2 to 0.13 ± 0.02 µg/ml when it was loaded into chitosan oligosaccharide micelles and the IC_{50} value of the drug had no significant change when chitosan oligosaccharide micelles were PEGylated [60]. In their previous studies on Caco-2 cells, Prego *et al.* [59] reported that toxicity of chitosan depends not only on its physicochemical properties but mainly on the concentration of the polymer exposed to epithelium. Additionally, they also found that chitosan nanocapsules have a dose-dependent cytotoxicity, 50% LD_{50} being around 1 mg/ml. On the contrary, current analysis results showed that PEGylated chitosan nanocapsules have a very good biocompatibility with the monolayers. More specifically, LD_{50} of chitosan–PEG nanocapsules was determined to be between 10 and 20 mg/ml. This indicates that the cytotoxicity inherent to chitosan nanocapsules was 10 – 20 times reduced owing to the PEGylation of chitosan [59].

Parveen and Sahoo [61] studied chitosan/PEG blended poly (lactic-co-glycolic acid) (PLGA) nanoparticles for tumor targeting drug delivery and they stated that PEG-modified nanoparticles had the lowest percentage of uptake by macrophages when compared with positively and highly negatively charged nanoparticles. Both *in vitro* and *in vivo* results showed that a combinatorial coating of PEG and chitosan led to a dramatic prolongation in blood circulation as well as reduced macrophage uptake with only a small amount of the nanoparticles sequestered in the liver. It was further established that the surface properties affect the cytotoxicity profile of the nanoparticles. PEG and chitosan coating significantly enhanced their cellular uptake and cytotoxicity in various cancer cell lines. Based on the results mentioned above, these stealth polymeric nanoparticles conferred by a combinatorial coating of PEG and chitosan may suffice for long circulation serving as an efficient targeted drug delivery system [61].

In conclusion, chitosan seems to have a potential for being safe for ocular applications [43].

4. Chitosan-based drug delivery systems for ocular application

Ocular application of chitosan formulations was studied by many researchers. Among the formulations are solutions [14,62], gels [5,15,18,63,64], liposomes [10], emulsions [39], nanoemulsions [25], NLC [7,23,65], micro- and nanoparticles [21,24,42,49,66,67], inserts [68] and chitosan conjugates [69].

4.1 Chitosan solutions

It is clear that increase in both the retention time in precorneal area and penetration of drug through the cornea are of great benefit in ophthalmic therapy [48]. A significant increase in the corneal residence time of tobramycin was obtained with chitosan solutions when compared with the commercial drug solution not only because of its ability to increase solution viscosity but also because of its mucoadhesive properties [14].

Topical application of chitosan solution has a penetration-enhancing effect as expected from polycationic chitosan derivatives soluble at physiological pH of the tear fluid [37,62]. This hypothesis was confirmed by preliminary results obtained with quaternary ammonium derivative *N*-trimethyl chitosan while *N*-carboxymethyl chitosan which is a polyanion at the physiological pH of tear fluid failed to enhance intraocular drug penetration significantly [62].

Efforts were oriented toward the addition of polyol salts bearing a single anionic head such as glycerol-, sorbitol-, fructose- or glucose-phosphate salts (polyol- or sugar-phosphate) to chitosan aqueous solutions. Proposed salts are ideal agents for transforming pH-dependent chitosan solutions into temperature-controlled pH-dependent chitosan solutions. Combination of chitosan, a cationic polysaccharide, and polyol-phosphate salt benefit from several synergistic forces favorable to gel formation including hydrogen bonding, electrostatic and hydrophobic interactions. Phosphate salts give chitosan a unique behavior by allowing chitosan solutions to remain liquid at physiological pH and turn into gel if heated at body temperature. Uniqueness of chitosan solution resides also

in overcoming pH barrier which has long been a major limitation for many applications. Thus, the phenomenon described here is quite distinct from that shown by solutions of water-soluble cellulose modified hydrophobically by ionic surfactants [70].

Gratieri *et al.* [5] investigated the ocular delivery of fluconazole by topical application of chitosan solution and poloxamer/chitosan *in situ* gel-forming formulation. The study demonstrated that poloxamer/chitosan *in situ* gel-forming formulation retarded fluconazole release when compared with the chitosan solution. However, chitosan solution was able to deliver higher fluconazole amounts than *in situ* gel-forming formulation in *ex vivo* studies on porcine cornea. Both formulations exhibited similar sustained release behavior in the *in vivo* study. It was concluded that both chitosan solution and *in situ* gelling formulation have the potential of treating fungal keratitis.

Solutions outstand with their simplicity in preparing and constitution with commercially available polymers at relatively low costs [5].

4.2 *In situ* gelling systems

In situ gelling systems can be easily and accurately applied in liquid form to the surface of the eye where crosslinkage occurs with the cations present in tear fluid in order to form a gel on the ocular surface. This is thought to prolong the precorneal retention time and thus lead to increased bioavailability of the drug [15].

Rapid turnover rate of the lacrimal fluid generally leads to a dilution of common viscous eye drops. Enhancement of the viscosity of formulations can be detected depending on the increase in the amount of cations present [15]. *In situ* gelling mechanisms can be triggered either by pH or by temperature changes [18,63,64,71]. Chitosan-based hydrogels can be formulated by the addition of a crosslinker to form covalent or ionic interaction between polymeric chains. Hydrogels can also be formed by complexation with another polymer generally by ionic interaction or by aggregation after chitosan grafting [72,73]. Covalently crosslinked hydrogels are the only systems characterized by a permanent network due to their irreversible chemical links. Therefore, they exhibit good mechanical properties and can overcome dissolution even in extreme pH conditions. On the other hand, other types of hydrogels are more labile. Covalent crosslinkers necessary to obtain hydrogels are either known to be relatively toxic or their fate in the human body is unknown and/or there is a lack of data concerning their biocompatibility. Therefore, an additional purification and verification step is required before administration of the hydrogel since it may be problematic if free unreacted crosslinker is found in traces before administration [72].

Gratieri *et al.* [18] prepared an *in situ* gel-forming formulation by combining poloxamer/chitosan and they obtained improved mechanical and mucoadhesive properties as well as enhanced retention time on mucin discs. Gels prepared proved to possess a mucoadhesive ability which is influenced by chitosan concentration. Gamma scintigraphic analyses in humans confirmed the prolonged retention time of the formulation due to faster gelling of the chitosan formulation under *in vivo* conditions which makes drainage more difficult. Delivery system developed seems to be a promising tool for ophthalmic use because it is easily administered and shows a prolonged ocular contact time [18].

Sustained ophthalmic drug delivery of baicalin over 8 h was obtained by pH-triggered *in situ* gelling system [74]. Formulation caused no irritation when tested on rabbit eye tissues. Both *in vitro* and *in vivo* results indicated that the pH-triggered *in situ* gelling system was a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through longer precorneal residence time and ability to sustain drug release. More importantly, gelling system can be considered as a novel ophthalmic delivery system being a suitable medium for pH-sensitive baicalin [74].

Physicochemical and rheological properties of a novel thermo-sensitive hydrogel system based on chitosan and inorganic phosphate was studied as a function of temperature [63]. There are two phase transition points as a function of temperature which corresponds to 30 and 43°C. While gel formation of the system at low temperature (< 30°C) was reversible, gel formation at high temperature (> 43°C) was irreversible. Referring to the results on pH value, conductivity and ionic strength changes as a function of temperature, it seems that hydrogen bonding between chitosan skeleton and water molecule and also the physical conjunctions may be the main driving forces to gel formation at low temperature (< 30°C). However, gel formation at high temperature (> 43°C) may be resulting from hydrophobic interactions [63].

4.3 Liposomes

Use of colloidal drug delivery systems such as liposomes is a suitable strategy to obtain enhanced ocular bioavailability in comparison with liquid formulations. Liposomes are preferred because they exhibit unique features by offering easy delivery, no interference with vision and stabilizing drug as an excellent reservoir [75]. On the other hand, liposomes are generally rather unstable and tend to degrade or aggregate and fuse which leads to the leakage of entrapped drug during storage or after administration. Among the many attempts aiming to minimize the disruptive influences is surface modification of liposomes which can improve liposomal stability both *in vitro* and *in vivo* [75].

It was reported that positively charged liposomes had a higher binding affinity to the corneal surface than the neutral and negatively charged vesicles as a result of interaction between positively charged liposomes and polyanionic corneal and conjunctival surfaces. However, cationic lipids such as stearylamine incorporated to give positive charge to liposomes may lead to irritation and toxic effects [10].

Formation of a bioadhesive and polymeric membrane around the liposomes was investigated [75]. Most of the selected membrane materials for liposomes were chitosan based [4,10]. Li *et al.* [10] studied low molecular weight chitosan-coated

liposomes and their potential use in ocular drug delivery. Researchers found that the coating layer added positive charge as well as an excellent bioadhesive property. Precorneal retention was significantly prolonged by chitosan coating compared with either non-coated liposome or drug solution. Chitosan coating also demonstrated an improved transcorneal drug penetration rate which was attributed to the penetration-enhancing effect of chitosan. Meanwhile, chitosan coating displayed preferable physicochemical stability and pronounced *in vivo* ocular tolerance [10].

Rational mixture of chitosan with phospholipids or with preformed liposomes led to formation of supramolecular hybrid structures [4]. These nanostructures demonstrated an adequate stability in biological fluids and are suitable for the encapsulation of labile macromolecules. They have an additional property of ability to control the release of entrapped molecules as a function of lipidic composition. It was shown that chitosan–lipid nanocomplexes can interact efficiently with ocular tissues and enter the ocular cells without compromising the integrity of cellular membrane.

Diebold *et al.* [76] analyzed the ocular application of liposome–chitosan nanoparticle complexes. Results of the study showed that nanosystems consisting of chitosan and phospholipids are first retained in the mucus layer and then enter the conjunctival cells at different levels depending on the composition. Furthermore, these nanosystems exhibited negligible toxicity *in vitro* and a good tolerance *in vivo* pointing out that liposome-chitosan nanoparticle complexes are promising candidates for drug delivery through the ocular mucosa [76].

4.4 Emulsions

Conventional ophthalmic dosage forms tend to be either simple solutions of water soluble drugs or suspensions of water insoluble drugs. Unfortunately, these delivery systems generally result in poor corneal drug absorption and therefore most of the drug applied does not reach the intended site of action. Microemulsions may offer a solution to the problem of poor corneal delivery by sustaining the release of the drug and also by providing a higher penetration into deeper layers of the eye. In addition, microemulsions have the potential of increasing the solubility of the drug in the vehicle [3].

In 2002, the Food and Drug Administration (FDA) approved the clinical use of an anionic emulsion containing 0.05% cyclosporine A (Restasis®, Allergan, Inc., Irvine, CA, USA) for the treatment of chronic dry eye. Either cationic or anionic nanoemulsions were recently approved for the treatment of ocular inflammations and for other ocular disorders [4].

Cationic emulsion was reported as being more effective in increasing the uptake of drugs in various ocular tissues following topical administration when compared with solutions or anionic emulsions [25].

Negatively charged emulsions can be prepared using anionic lipids and surfactants while positively charged emulsions using cationic lipids such as stearylamine and oleylamine. Alternatively, cationic polysaccharides such as chitosan can be used to form a coating around the oily droplets thus impairing a positive charge to the emulsion [4]. Unfortunately, stearylamine showed *in vitro* high toxicity against the tested cell systems [1,10]. Cytolytic and cytotoxic activity limits the consideration of these systems as novel drug delivery carriers [77].

Chitosan has proved to be a useful emulsifier that stabilizes emulsions and prevents coalescence by steric and electrostatic hindrance without the help of additional surfactant due to its self-cationic character [4].

Drugs incorporated into oil-in-water emulsion (o/w) are lipophilic in nature and either corneal or conjunctival/sclera route of penetration is favored depending on the extent of lipophilicity [39].

4.5 Particular systems

Micro- and nanoparticles were shown to be efficient ocular delivery systems [22,27,28,49]. Since composition of the colloidal system may affect its affinity to the ocular mucosa, several approaches were investigated for the ultimate formulation [22,28,48,49,66].

Microspheres have the potential of being used for targeted and controlled release drug delivery. Addition of bioadhesive properties to microspheres has additional advantages, for example, efficient absorption and enhanced bioavailability due to a much more intimate contact with the mucus layer by high surface to volume ratio, and specific targeting of drugs to the absorption site [20].

Bioadhesive microspheres can be tailored to adhere to any mucosal tissue thus offering the possibility of localized or systemic controlled release systems. Application of bioadhesive microspheres to the mucosal tissues of ocular cavity is used for administration of drugs mostly for local action [20].

Prolonged release of drugs and a reduction in frequency of ocular administration can highly improve patient compliance [20].

Two approaches regarding ocular application of chitosan particles are incorporation of active agent into chitosan nano- and microparticles or chitosan-coating of either polymeric or lipidic particles [20,21,66].

In a study by Yuan *et al.* [66], ocular application of rapamycin-incorporated chitosan nanoparticles and rapamycin suspension was compared. Ocular distribution results showed that both formulations showed good spreading characteristics over the entire precorneal area just after topical administration. Animals treated with rapamycin-incorporated chitosan nanoparticles presented significantly higher ($p < 0.05$) remaining radioactivities on corneal and conjunctival surfaces than those treated with rapamycin suspension (two to six times increase; for at least 24 h). Enhanced duration on ocular surfaces was attributed to the mucoadhesive character of chitosan mediated by the electrostatic interaction between positively charged chitosan and negatively charged corneal and conjunctival cells. They concluded that chitosan formulations can improve the residence

time of drug on tissues and cells, release the drug in a sustained pattern and thus improve the bioavailability of drug and reduce the administration frequency [11,51,66].

De Campos *et al.* [21] investigated the potential use of chitosan and chitosan-coated nanoparticles for specific delivery of drugs to the ocular mucosa. They considered the advantages of intimate contact tendency with the corneal and conjunctival surfaces, increasing delivery to only external ocular tissues and maintaining long-term drug levels. Systems showed great promise with at least 24 h corneal and conjunctival residence time [21,49].

Mucoadhesive chitosan–sodium alginate nanoparticles were investigated as a new vehicle for prolonged ophthalmic delivery of an antibiotic, gatifloxacin [67]. Analyses results showed that the drug was released from the optimized formulation over a period of 24 h in a sustained release manner primarily by non-Fickian diffusion. Formulation prepared was proposed to be a viable alternative to conventional eye drops by virtue of its ability to sustain drug release, ease in administration and reduced dosing frequency resulting in better patient compliance.

The second generation of submicron particles, NLC can also be used as topical drug delivery system for ocular mucosa [65,78]. NLC combines many features of pharmaceutics, that is, prolonged release of actives, drug targeting and increasing amount of drug penetrating into mucosa. NLC exhibits an excellent tolerability due to the physiological and/or biodegradable lipids used in the formulation [23]. Studies indicated that NLC increases the ocular bioavailability of lipophilic drugs without inducing discomfort or irritation [7,79]. Resulting findings of prolonged precorneal residence time and delivery to ocular surface and anterior chamber showed that thiolated NLC is a promising strategy to the treatment of ocular surface and anterior segment inflammatory diseases (e.g., uveitis) [65].

When the potential of chitosan-coated NLC was investigated for ocular delivery, it was found that positive charge of NLC dispersions provided a longer retention time by interacting with the negatively charged mucous. Eventually, an improved penetration rate was achieved by the presence of chitosan concerning its effective contribution to the corneal permeability. The most notable advantage of chitosan-coated NLC was their superior mucoadhesive properties [23].

Existence of the bioadhesive polymer chitosan on nanocapsules was concluded to provide an optimal corneal penetration of encapsulated drugs with good ocular tolerance. Chitosan-coated colloidal drug carriers were proposed as promising systems to overcome the present limitations in ocular drug delivery [23,24,48].

4.6 Other delivery systems

Films, erodible and non-erodible inserts, rods and shields are the most logical delivery systems aiming the long remaining time on ocular surface. These delivery systems sustain and control drug release and thus avoid pulsed entry characterized by a transient overdose, followed by a relative short period of acceptable dosing which in turn is followed by a prolonged period of low dosing [13].

Mono- and bilayer dexamethasone-chitosan films were successfully obtained and their release tests suggested that the films are potential sustained-release carriers for dexamethasone. Incorporation of a second layer of chitosan film modified drug release profile significantly. As a conclusion, while the monolayer dexamethasone-chitosan film is promising for dexamethasone for a few hours, bilayer dexamethasone-chitosan film seems to be promising for weeks [80].

Di Colo *et al.* [68] prepared an insert aiming enhanced ocular bioavailability of ofloxacin. Following insertion, every insert formed a superficial gel, adhered to the application site and then gradually spread over the cornea and eroded. While remarkable bioavailability increase was determined compared with commercial eye drop, signs of mild irritation were seen [68].

5. Expert opinion

Topical ocular route of administration is preferred for many drugs due to ease in access and high patient compliance when treating diseases at both anterior and posterior segments. Providing a sufficient dose at the desired site of action is a great challenge for ocular therapeutics due to anatomic and physiologic barriers of the eye limiting drug delivery especially to the posterior segment tissues. Most common approaches for the enhancement of ocular bioavailability are prolonging retention time and enhancement of ocular penetration. Cationic lipids used widely for those purposes were limited by their cytotoxic effects. As an alternative, self-cationic polymers like chitosan gained more attention in ocular applications. Several types and derivatives of chitosan can be tailored from chitin leading to a possibility of selecting the most appropriate chitosan type to obtain the desired characteristics of delivery systems for both hydrophilic and lipophilic drugs.

Aiming enhanced ocular bioavailability, different drug delivery systems are as important as the polymeric structure. Due to the transcendent properties of the polymer, many formulations were developed using chitosan. Among those, particulate systems seem to be the most promising system for ocular applications. Considering the superior characteristics of particulate systems like enhanced stability of the drug incorporated, perdurable particulate structure, high drug payload, controlled release of the actives, etc., incorporation of chitosan into those systems contributes to the properties mentioned.

Emulsification/solvent evaporation, spray-drying, ionotropic gelation and coacervation techniques using chitosan results in nano/microparticulate systems while avoiding organic solvents and preventing coalescence caused by steric and electrostatic interactions owing to chitosan's emulsifying character, possibility of incorporating both hydrophilic and lipophilic drugs which makes chitosan more preferential.

PEGylation must be taken into account when safer and more effective particulate formulations are required. Either

chitosan-based or chitosan-coated particles can be PEGylated resulting in improvement of *in vitro–in vivo* stability, decrease in toxicity and enhancement of ocular penetration due to enhanced mucoadhesive characteristics of the nanocarriers.

Chitosan itself also enhances the penetration of drugs by opening the tight junctions between epithelial cells or by intracellular routes. Chitosan provides the drug to enter the ocular cells without disturbing the integrity of cellular membrane and further PEGylation leads to more enhanced mucoadhesion. Since nature of coating affects the interaction with the epithelial cells and also transport across the corneal epithelium, precise concentrations are required.

Overviewing the outstanding features of chitosan, it seems to gain increasing attention in the treatment of severe ocular disorders.

Declaration of interest

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

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