*Teaser Successful drug delivery to ocular targets depends on ocular biology, drug properties, and formulation characteristics. A multifactorial design aid for ocular drug delivery is presented.*

# Design principles of ocular drug delivery systems: importance of drug payload, release rate, and material properties

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**Ocular drugs are usually delivered locally to the eye. Required drug loading, release rate, and ocular retention time of drug delivery systems depend on the potency, bioavailability, and clearance of the drug at the target site. Drug-loading capacity of the formulation is limited by the material properties and size constraints of the eye. The design aid described herein for ocular drug delivery systems guides the calculation of steady-state drug concentrations in the ocular compartments, taking into account drug dose, bioavailability, and clearance. The dosing rate can be adjusted to reach the target drug concentrations, thereby guiding the design of drug delivery systems for topical, intravitreal, and subconjunctival administration. The simple design aid can be used at early stages of drug development by investigators without expertise in pharmacokinetic and pharmacodynamic modeling.**

## Introduction

Most ophthalmic treatments of anterior segment diseases rely on eye drop medication that is administered in outpatient settings. Although the instillation of eye drops appears to be an easy procedure, many patients, especially those with compromised eyesight [1], experience many difficulties in their administration. In addition, because the duration of drug action is relatively short, frequent drug administration, often one to eight times daily, is needed [1]. Therefore, patient compliance is often low; for example, in glaucoma only ~50% of patients use their medication properly [2]. Topical, patient-friendly, and long-acting delivery systems are needed as anterior segment treatments. Furthermore, topically delivered medication does not reach drug targets in the posterior segment, whereas retinal diseases are treated with intraocular injections.

Drug delivery to intraocular tissues can be improved with intraocular injections and implants, but they must be given by ophthalmologists and specialized nurses. Intravitreal delivery is the

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only clinical option in the treatment of posterior segment diseases (e.g., of the retina and choroid). The burden of such injections to patients and the healthcare system is enormous. For example, nearly 20 million anti-vascular endothelial growth factor (VEGF) injections are given intravitreally per year to treat wet age-related macular degeneration (wAMD) (Market Scope Estimate http://market-scope.com/). These injections should be given at intervals of 1–2 months, but in practice the injection intervals are longer. Other routes of drug administration (e.g., subconjunctival or suprachoroidal) and prolonged action formulations have been investigated as alternatives to intravitreal injections [3]. New drug delivery systems, less invasive or longer acting, are needed for posterior segment treatments.

Ocular drug targets are located in anatomically distinct regions in the anterior or posterior tissues of the eye. The efficacy of the treatment depends on the disease state, drug properties, and delivery to the target sites. For each route of ocular drug administration, drug delivery depends on the pharmacokinetic key parameters, such as bioavailability, drug elimination from the target tissue, dosing regimen, and release and/or dissolution of the drug.

Here, we present a simple, systematic, guidance for ocular drug dosing and delivery system design. We integrate ocular pharmacokinetics (e.g., site of administration, bioavailability, and clearance), and drug potency (required concentrations at the target site) together with formulation properties (retention at application site, drug payload, and release rate). This multifactorial approach allows us to generate a quantitative simple framework that will help in the choice of doses, release rates, and materials for ocular drug delivery systems without the need for expertise in pharmacokinetic or pharmacodynamic modeling.

## Topical application

### *Drug targets and pharmacokinetics*

Topical drug delivery is accomplished by applying the drug product to the ocular surface, where it mixes with the lacrimal fluid. This mode of drug delivery is used to treat anterior segment diseases affecting the ocular surface (e.g., dry eye disease or infections), that is, the cornea and conjunctiva (infection, inflammation, or neovascularization) or tissues surrounding the anterior chamber (e.g., elevated intraocular pressure, inflammation, or infection) (Fig. 1). To treat the ocular surface, the drug must be retained in the tear film (e.g., treatment of dry eyes) or absorbed by the cornea or conjunctiva (e.g., treatment of keratitis or conjunctivitis). In the case of intraocular target tissues, such as the trabecular meshwork, iris, or ciliary body, the drug must permeate across the cornea and/or conjunctiva to reach these tissues [4]. For example, prostaglandins reduce intraocular pressure in glaucoma primarily by acting in the trabecular meshwork, whereas beta-blockers and carbonic anhydrase inhibitors act in the ciliary body [5,6]. Topical ocular delivery does not typically result in effective concentrations in the posterior segment [7].

Most clinically used topical drugs permeate across the cornea to the aqueous humor (Fig. 1) [8]. The corneal epithelium is the major barrier to drug absorption, whereas drug diffusion in the corneal stroma and endothelium is unrestricted [9]. From the aqueous humor, drugs distribute easily to the trabecular meshwork, iris, and ciliary body. However, the physical lenticular barrier, blood flow of the iris–ciliary body, and aqueous humor turnover limit drug distribution further to the vitreous and retina [4]. Small-molecule drugs are eliminated from the anterior chamber through the aqueous humor outflow and blood flow of the iris and ciliary body at clearance rates of 5–35 μl/min [10,11]. Large molecules are cleared only via aqueous humor outflow (~2.4–5.2μl/min) [10].

Topical drugs can be absorbed from the ocular surface across the conjunctiva and sclera to the iris and/or ciliary body without first entering the aqueous humor (Fig. 1) [8,12,13]. This route is important for the absorption of hydrophilic small molecules, and a viable option for large molecules, because the intercellular spaces in the conjunctival epithelium are wider than in the cornea, being more permeable to larger molecules.

The bioavailability of topically applied ocular drugs in the aqueous humor is usually in the range of 0.001–0.05 (i.e. 0.1–5%) and limited by several factors. First, the short retention of eye drops without viscosity enhancers on the ocular surface limits corneal drug absorption. For example, regular eye drops flow from the ocular surface to the nasal cavity in a few minutes [14]. Second, drug absorption across the conjunctiva and into the blood stream is fast; for example, ~50% of instilled pilocarpine is absorbed from the lacrimal fluid directly into the blood circulation [15]. Systemic absorption from the palpebral conjunctiva (lining the inner side of eye lids) further decreases drug concentration in the tear fluid [7], whereas systemic drug absorption in the bulbar conjunctiva (lining the ocular surface) also limits drug permeation deeper to the sclera and ciliary body [12]. The conjunctival surface area is an order of magnitude larger than that of the cornea [16], partly explaining the extensive trans-conjunctival drug flux. Third, the intercellular tight junctions on the surface of the corneal epithelium limit absorption of small molecules and block the permeation of macromolecules, such as proteins (Table 1) [17,18].

Small molecules permeate across the cornea and conjunctiva by passive diffusion (trans-cellularly and/or paracellularly). Many transporter proteins, such as P-glycoprotein and multidrug-associated proteins (MRP-1 and MRP-4), are expressed in the cornea, but their influence on ocular drug bioavailability remains unclear [19]. Corneal permeation of small molecules can be predicted *in silico* from their physicochemical properties, such as logD, hydrogen bonding, and polar surface area [20]. However, increased corneal drug permeability is associated with increased trans-conjunctival drug elimination to the systemic circulation. Therefore, even in the best cases, drug bioavailability in the anterior chamber is only ~0.05 [21]. For example, timolol concentrations in the cornea, aqueous humor, and vitreous are 0.4–4%, 0.2–0.6%, and 0.002–0.01%, respectively, of the timolol concentrations in the eye drop, illustrating the roles of physical barriers and clearance mechanisms in ocular pharmacokinetics [7].

Currently, only small-molecule drugs are applied topically in clinical ophthalmology. Nevertheless, conjunctiva and sclera allow permeation of hydrophilic and large molecules: in the conjunctiva, compounds with molecular weights up to 5 kDa are able to permeate, whereas the sclera allows passage of macromolecules (e.g., molecular weight of 100 kDa) (Table 1) [17,22].

### *Dosing considerations*

The required drug dosing during chronic drug administration can be estimated using Eq. (1) [33]:

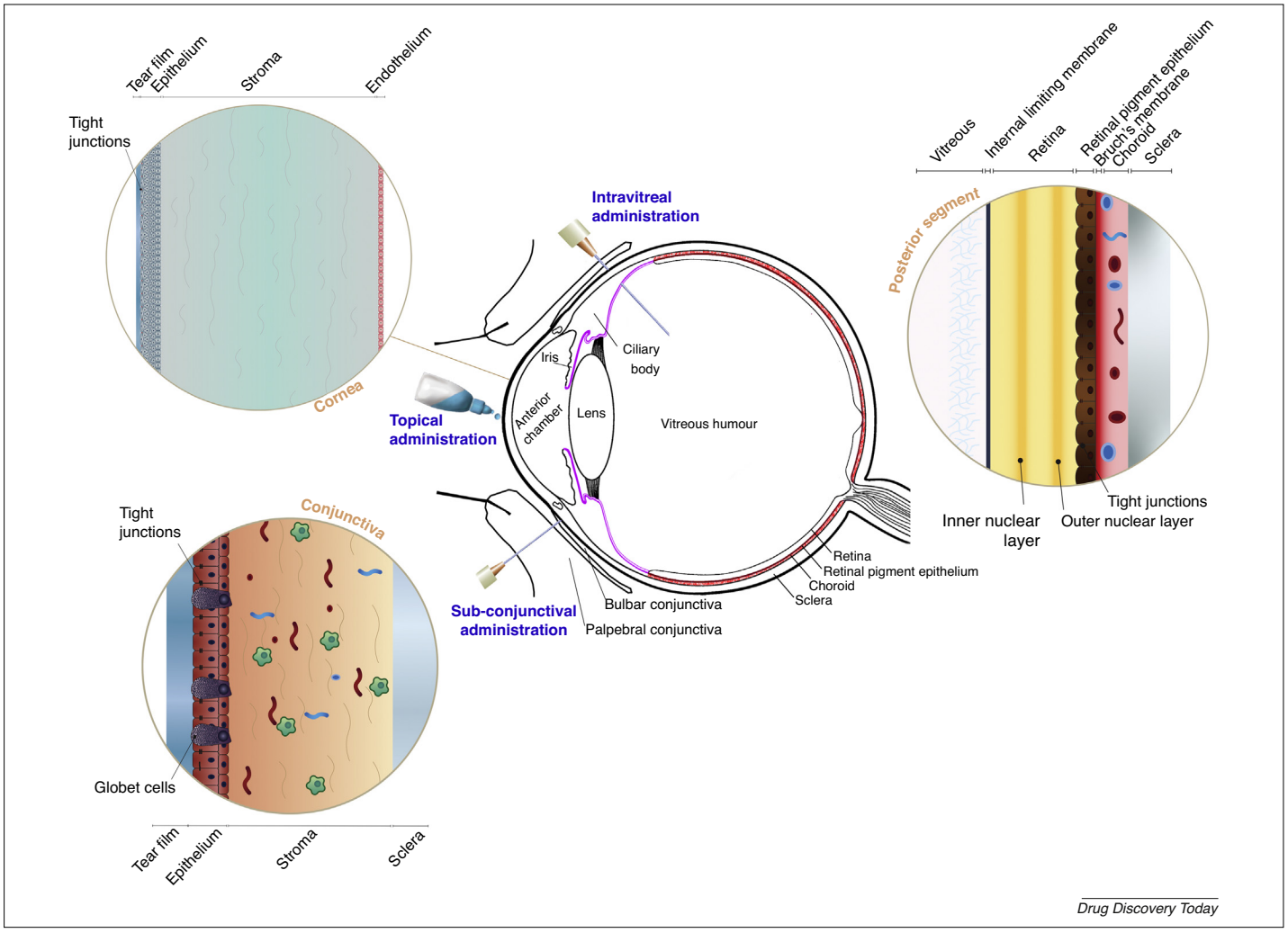


**FIGURE 1**

Cross-section of the eye and routes of drug administration (topical, subconjunctival, and intravitreal). The barriers for drug penetration after topical, subconjunctival and intravitreal delivery are shown in the zoomed pictures.

$$D = C_{ss,av} \times CL \times \tau/F \quad (1)$$

where D is the drug dose in the formulation, $C_{ss,av}$ is the average steady-state concentration at the target site, CL is the drug clearance from the target compartment (in the case of topical application, the aqueous humor, $CL_{AH}$), $\tau$ is the dosing interval, and F is the bioavailability (i.e., the fraction of the drug dose that reaches the target compartment, such as the aqueous humor, $F_{AH}$). Typical values of $CL_{AH}$ and $F_{AH}$ range from 5 to 35 μl/min and 0.005 to 0.05, respectively [34]. They can be used to calculate the required daily drug doses for compounds to reach a certain target concen-

**TABLE 1**

**Physical tissue barriers in the eye**

| Ocular barrier | Thickness in humans (μm) | Pore size (nm) (species) | Barrier properties | Refs |
|---|---|---|---|---|
| Corneal epithelium | 52 | 2 (rabbit) | Multilayered epithelium with tight junctions in outermost layer; small molecules can permeate | [23] |
| Conjunctiva | 42 | 3–5 (rabbit) | Multilayered epithelium with tight junctions in outermost layer; small and large molecules (up to 5 kDa) can permeate | [23–25] |
| Sclera | 400–900 | 10–50 (rabbit) | Porous supporting tissue (collagen and polysaccharide fibers) that allows permeation of macromolecules and small nanoparticles | [26] |
| Vitreous | ~15 000 | 500 (bovine) | Gel-like network; small drugs, proteins, and nanoparticles can diffuse | [27,28] |
| ILM | 0.05–2.00 | 10–100 (human) | Hydrophilic acellular basement membrane; molecules and small nanoparticles can permeate | [29,30] |
| RPE | 26 (including Bruch's membrane) | 2 (bovine) | Cell monolayer with tight junctions. Small lipophilic compounds penetrate faster than hydrophilic or large molecules | [31,32] |

tration ($C_{ss,av}$). The required drug doses (D) for different dosing intervals (τ) were calculated for hypothetical drugs with target $C_{ss,av}$ in the aqueous humor (1 nM–100 μM) (Fig. 2a). These calculations were performed assuming a molecular weight of 500 g/mol, $CL_{AH}$ = 10 μl/min and F = 0.02.

The strong dependence of dosing levels and drug potency is obvious; for example, for the target concentration of 100 μM in the anterior chamber, the daily drug dose must be >30 mg (Fig. 2A), which is approximately the same as the weight of the entire eye drop (30–40 mg). At $C_{ss,av}$ = 10 nM, the daily dose is only 3.6 μg (e.g., 0.012% eye drop, 30 μl) and $C_{ss,av}$ = 1 μM is reached with a daily dose of 0.36 mg (30 μl of 1.2% eye drop). In the case of a 10 μM target concentration, a 12% solution should be used, which is rarely possible for solubility and tolerability reasons. Alternatively, five to ten eye drops should be instilled daily at 1–2-h intervals. Many drug candidates have poor solubility and, therefore, are formulated as suspensions. In such cases, the amount of soluble drug in the suspension drop should be used as the dose to estimate the minimum reachable value for $C_{ss,av}$. A small fraction of the solid drug might dissolve during the contact time on the ocular surface [35]. An estimate of the maximal absorbable dose is only approximately five times the soluble dose, although the drug quantity in the particles can be one to two orders of magnitude higher than the quantity of dissolved drug [35,36].

In the case of long-acting delivery systems (e.g., microspheres, gels, and implants), drug loading and release must result in therapeutic drug concentrations during the entire dosing interval (e.g., days or weeks). For a topical drug delivery system with dimensions of 1 mm × 1 mm × 5 mm (weight ~5 mg), a drug loading of 10% would mean a drug dose of 0.5 mg. Thus, the target concentration should be 0.1 μM or less for dosing intervals of days to 2 weeks (Fig. 2a). Longer acting delivery systems are suitable only for potent drugs ($C_{ss,av}$ ≈ 0.01 μM or less). Given the physicochemical factors, the loading capacity of 10% is not always possible. Obviously, a loading capacity of 1% in a 5 mg device would result in even more stringent requirements on drug potency ($C_{ss,av}$ ≈ 10 nM for <2 weeks dosing intervals; 1 nM for longer dosing

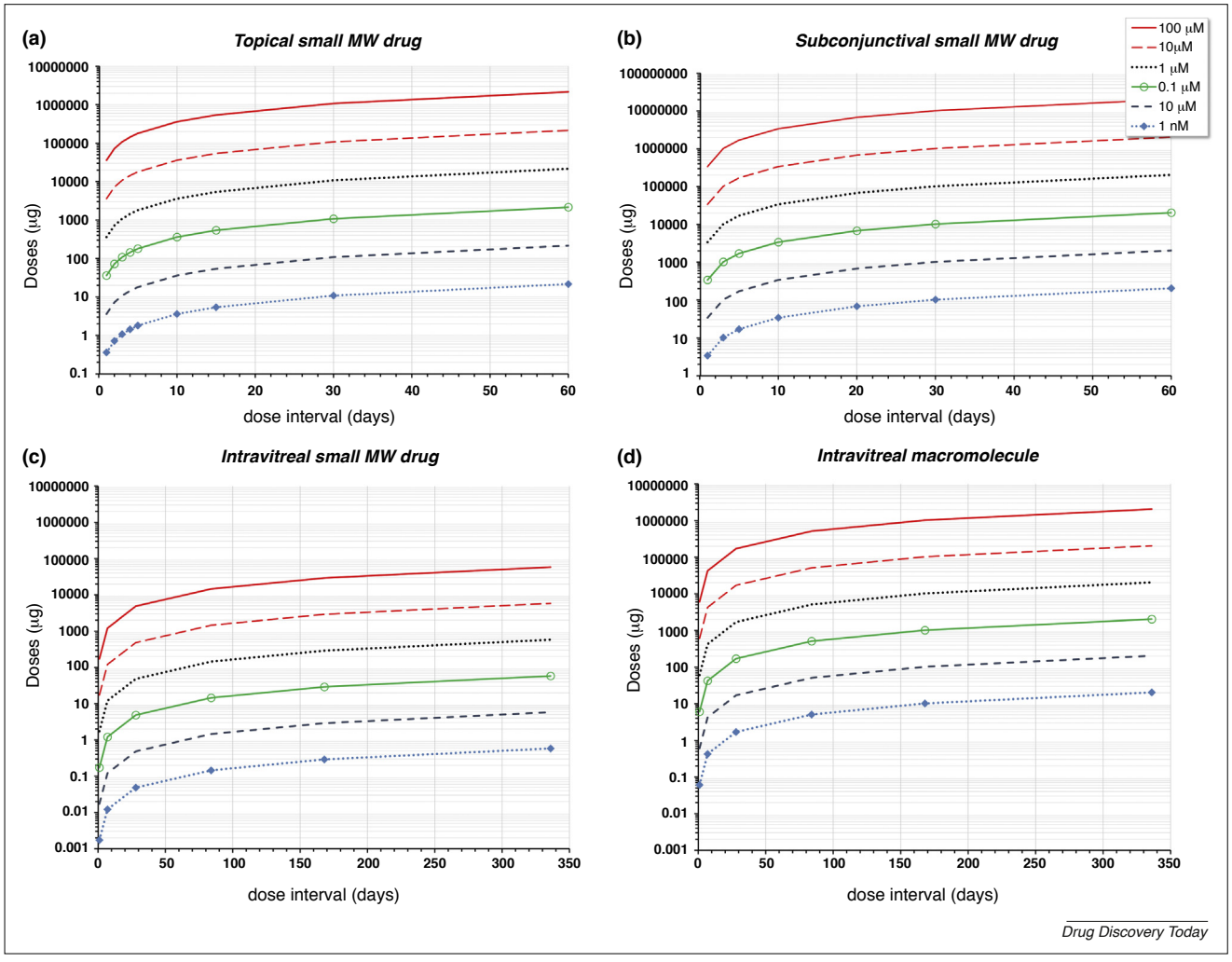


**FIGURE 2**

Drug-dosing requirements as a function of the dosing interval. The calculations are presented for compounds with target concentrations of 1 nM (blue-dotted line), 10 nM (black-dashed line), 100 nM (green line), 1 μM (black-dotted line), 10 μM (red-dashed line) and 100 μM (red-solid line). **(a)** Topical small molecule with target concentrations in the aqueous humor of F = 0.02 and $CL_{AH}$ = 10 μl/min. **(b)** Subconjunctival small molecule. Target concentration in the retina and vitreous humor of F = 0.001 and $CL_V$ = 4.7 μl/min. **(c)** Intravitreal small molecule with target concentrations in the vitreous humor of F = 1.0 and $CL_V$ = 4.7 μl/min. **(d)** Intravitreal large molecule. Target concentration in the vitreous humor of F = 1.0 and $CL_V$ = 1.1 μl/min.

frequencies). The higher the drug potency, the easier the drug payload requirements.

Ocular bioavailability will increase with prolonged retention of the delivery systems on the ocular surface. However, systemic conjunctival absorption will limit the maximum values of $F_{AH}$ to ~0.05–0.1, even at high corneal permeability values [7,21]. Corneal drug permeability can be obtained from the literature or predicted *in silico* and further used to estimate $F_{AH}$ [20,21]. If such data are not available, then 0.01–0.05 and 5–35 μl/min can be used as the ranges for $F_{AH}$ and $CL_{AH}$, respectively, for initial drug dosing calculations with Eq. (1).

### *Drug delivery system design*

A topically applied drug delivery system will be in contact with the tear fluid and ocular surface tissues. The tear fluid (7 μl) forms a thin layer comprising three parts: the outermost lipid layer (200 nm), the aqueous layer (3–7 μm) with secreted mucins and other soluble proteins, and the mucin-containing gel layer (1 μm) [37]. The protein content of the tear fluid is lower than in plasma, but it still contains lysozymes that protect the eye from invading microbes by breaking 1,4-glycosidic bonds [38]. The tear fluid is weakly buffered by bicarbonate and its normal pH of ~7.4 can be transiently changed after eye-drop instillation [39].

Cell surface mucins form a hydrophilic glycocalyx, but the role of this layer as a permeation barrier is not well established [40]. Hydrophilic polymers, such as polyethylene glycol, polyvinylpyrrolidone, polyvinyl alcohol, and chitosan, bind to the mucins with hydrogen bonds and/or electrostatic interactions [41]. Mucoadhesion prolongs the contact of the formulation on the ocular surface: this is an important prerequisite for improved ocular drug bioavailability and duration of drug action. Nevertheless, prolonged duration of action also requires controlled drug release from the delivery system, in addition to long retention.

Table 2 presents selected clinical and experimental drug delivery systems. The reliability of Eq. (1)-based calculations can be tested with these data. Take as an example the solid polymeric insert that releases 0.75 mg of ciprofloxacin (331.34 g/mol) in 5 days and yields a $C_{ss,av} = 1$ μM [42]. Using standard values $F_{AH} = 0.02$ and $CL_{AH} = 10$ μl/min, we can calculate that a dose of 1.19 mg/5 days is needed to reach a 1 μM steady-state concentration in the aqueous humor. In another study, diclofenac (296 g/mol; dose 50 μg) was delivered from polymeric micelles and hydrogels to rabbit eyes, resulting in ~1–4 μM drug concentrations in the aqueous humor [43]. Using standard values of $CL_{AH}$ and $F_{AH}$ and a dosing interval of 8 h, we can estimate that doses of 90–360 μg are needed to reach a $C_{ss,av}$ of 1–4 μM. A pilocarpine (208 g/mol) insert (Ocusert) releases drug at 20 μg/h [44]. At this release rate, the steady-state pilocarpine levels in aqueous humor are calculated to be 0.66 μg/ml, whereas levels of 0.4 ± 0.1 μg/ml were determined experimentally in rabbit eyes [43]. These simple calculations provide reasonable estimates, easily within the same order of magnitude, for the required dosing levels. Such calculations will help to reject any early-stage drug development projects that do not have chances of success (i.e., because of nonfeasible dosing requirements).

These calculations provide an early guidance to the required drug loading in the delivery system based on the drug potency, pharmacokinetics, and intended dosing interval. There are various polymeric and lipid materials that have been tested experimentally and/or used topically in clinical ophthalmology. Several criteria must be met in the delivery system design, including safety, industrial manufacturing, stability, and sterility. Herein, we briefly discuss the biopharmaceutical factors that are required for successful delivery: drug dose and loading, drug release, and retention. The discussion is focused on small-molecule drugs, because the topical delivery of biologics is not considered to be feasible.

Drugs can exist in the delivery system either in the dispersed or dissolved form. Dispersed drugs are not dissolved in the material but exist as solid particles within the carrier material. This is a common approach for oral drug formulations, but in ophthalmology this approach is used only for suspensions of poorly water-soluble compounds, such as fluorometholone. In those cases, drug

**TABLE 2**

**Examples of topical ocular drug delivery systems**

| Material | Drug | Drug loading (μg/mg)/dose (mg) | Release time (*in vitro*) | Retention in conjunctival sac (h) | Concentration range (μM in aqueous humor) | Refs |
|---|---|---|---|---|---|---|
| **Liquid formulations** | | | | | | |
| Solid-lipid nanoparticles | Indomethacin | 50/5.0 | | | ~1.0–2.5 | [53] |
| Mesoporous silica (suspension) | Dexamethasone | 29–68/0.35 | 12 h (burst release) | | 0.12–1.10 | [54] |
| Montmorillonite/chitosan | Betaxolol hydrochloride | 14/1.4 | 4 h (burst release) | 1.5 | 3–23 | [55] |
| PLA-PEG | Cyclosporine | ~65/3.0 | 2 h | 1 | 16–25 | [56] |
| Timoptol XE (gellan gum) | Timolol | 2.5/0.125 | 6 h | | 0.6–12 | [57] |
| **Semi-solid formulations** | | | | | | |
| Pluronic F127 | Timolol maleate | 5/0.25 | 4 h | n.d. | 3–28 | [48] |
| Methoxy poly(ethylene glycol)/cyclodextrin | Diclofenac | 1/0.05 | 48 h | 6 | 1–8 | [43] |
| Solid formulations | | | | | | |
| Eudragit or polyvinyl acetate film with sodium alginate core | Ciprofloxacin hydrochloride | 750 μg/insert | 5 days (zero-order release) | 120 | 1 | [58] |
| Ocusert (ethylene vinyl acetate) | Pilocarpine | 5/11 mg | 1 week (20–40 μg/h) | 168 | 2 | [59] |

dissolution might partly control ocular drug delivery instead of the corneal absorption. Therefore, after application of a suspension to the lacrimal fluid, the ocular drug exposure increases only modestly compared with the application of a saturated solution, and only modest increases in duration of drug action are seen. Optimized delivery from suspensions would require systematic efforts to tune the drug dissolution time with matching particle retention in the lacrimal fluid: small particles dissolve faster, but they are removed more quickly from the ocular surface [45]. The upper limit of the particle size (diameter of 10 μm) has been set to avoid any foreign body sensation and reflex tearing.

In most topical ocular drug delivery systems, the drug is dissolved in a matrix material, such as a polymer, and its solubility depends on the properties of the drug and the matrix. For example, lipid soluble and nonpolar drugs (e.g., latanoprost or betaxolol) partition well into nonpolar environments, such as lipids and hydrophobic polymers [e.g., poly(lactic-glycolic acid, PLGA)] [46]. The loading of hydrophilic drugs (e.g., ciprofloxacin and acetazolamide) to such materials instead is possible only in very small amounts. Furthermore, loading in disperse systems is limited, because the volume of the dispersed phase (e.g., nanoparticles, liposomes, or microspheres) is only a small fraction of the formulation. Only the dispersed phase is available for controlled-release purposes; for example, a 10 mg/ml polymeric dispersion contains only 0.3 mg of polymer phase in a 30 μl eye drop. This is a serious limitation and such systems might be suitable only for potent drugs with target concentrations in aqueous humor of 10 nM and less (Fig. 2, Table 1).

Conventional nonviscous eye-drops are retained only for a few minutes on the ocular surface. The retention can be prolonged with viscosity increasing and mucoadhesive polymers, which improve ocular bioavailability and peak concentrations in the aqueous humor (up to twofold) without exerting any control on drug release [47,48]. Controlled release without extended retention on the ocular surface is not useful: many liposomal and suspension formulations have short preocular contacts of only a few minutes, but they release and/or dissolve drug only slowly, resulting in poor ocular bioavailability [49]. Therefore, the delivery system must be retained on the ocular surface long enough to release the drug dose in the lacrimal fluid. Retention on the ocular surface can be extended with polymers that show mucoadhesion based on electrostatic interactions or hydrogen bonding. By contrast, the physical size of the delivery system has an important role: without mucoadhesion, nano- and microparticles are quickly cleared from the ocular surface, but contact lenses, gels, and solid inserts can be retained on the corneal surface or in the conjunctival sac for days, even weeks.

Drug release and dissolution on the ocular surface is a crucial issue in reaching the desired steady-state concentrations in the aqueous humor, because only the released or dissolved drug can be absorbed into the eye. Doses on the y-axis in Fig. 2a refer to the dose that is available for absorption. If the drug is drained away from the ocular surface before it is released, that part does not contribute to the dose. For example, if 90% of the dose is lost from the ocular surface before drug release, the bioavailability will decrease by 90% from the assumed levels in Fig. 2A. Drug release from the material can be controlled in many ways. For example, hydrophobic interactions, electrostatic interactions, π–π interactions, and hydrogen bonding can be used to increase drug affinity to the matrix and slow the release rate [50,51]. By contrast, the mesh size in the materials (e.g., gels) can be tuned with crosslinking and interchain affinity in the polymer. Finally, the release rate can be adjusted based on the biodegradation rate of the polymer (e.g., PLGA). Relevant release rates ranging from hours to weeks can be achieved with nanoparticles, microspheres, gels, and inserts (Table 2).

In dispersed drug formulations, a drug will equilibrate between the dispersed particles and the aqueous solution that forms most (usually >95%) of the total volume. The storage times of such systems can be months, even years, whereas the release times on the ocular surface are only hours, days, or weeks at a maximum. Premature drug release from the dispersed particles is a major risk and can be avoided only if the equilibrium is almost completely on the side of particle-bound state. In that case, the drug should be released from the dispersed particles during retention in the lacrimal fluid. In principle, freeze drying can be used to avoid drug release during the shelf-life, but this is not a practical solution from an industrial viewpoint. Multidose eye-drop bottles are used for 1 month, and dissolving powder for every instillation from single-dose containers is not a viable option. This limitation is not crucial for gels, contact lenses, or inserts, because they do not have two phases and, thus, the associated risk of drug release during storage is minimal.

Drug release is normally tested *in vitro* using 'sink conditions'; that is, the drug concentration in the release medium remains negligible (<10% of its concentration in the formulation). This is achieved by using large volumes of the release medium (leading to extensive drug dilution) or a flow-through system that removes the released drug. Drug release in the tear fluid takes place in a tiny volume (7 μl), but the clearance is relatively high (~10 μl/min) because of the clearance across the conjunctiva to the blood circulation. Release comparisons between *in vitro* and *in vivo* situations have been done only in a few cases [42,52]. For instance, timolol release from silicone capsules was similar *in vitro* and *in vivo*, because rapid drug clearance from the tear fluid maintained drug levels at low concentrations, generating sink conditions. Based on the dosing considerations above, the relevant topical drug release rates are <100 μg/day (i.e., ~4 μg/h). At such release rates, the expected drug concentrations in the tear fluid are <6 μg/ml; low concentrations that should be compatible with sink conditions.

## Intravitreal injection

### *Drug targets and pharmacokinetics*

Drug targets depend on the disease and drug type. For example, wAMD is treated with intravitreal injections of biologics that inhibit extracellular VEGF in the retina and vitreous [5]. The more common dry AMD is characterized by impaired autophagy, oxidative stress, and inflammatory responses in the retinal pigment epithelium (RPE), a potential cell target. Diabetic retinopathies and diabetic macular edema are treated with anti-VEGF agents and corticosteroids with extracellular and intracellular target proteins, respectively. Other potential target sites include photoreceptors (retinal degeneration), ganglion cells (glaucoma), and optic nerve (glaucoma).

Drug targets in the posterior eye segment can be reached with direct intravitreal injections (Fig. 1). After diffusion in the vitreous,

the injected drugs are eliminated via anterior and posterior routes [4]. Posterior clearance results in rapid elimination from the vitreous (half-lives in the range of hours), whereas only anterior clearance results in half-lives of days [60]. Posteriorly, drugs permeate across the blood ocular barriers in the retina (RPE and endothelia of retinal capillaries) and iris–ciliary body. These barriers have intercellular tight junctions that limit the permeation of large molecules, whereas small and particularly lipophilic compounds will pass through the barriers up to 50 times faster [31]. Anterior elimination involves drug diffusion from the vitreous to the aqueous humor (Fig. 1). The size of this route is limited by the iris–ciliary body and the lens (Fig. 1). Drugs are eliminated from the aqueous humor with the outflow into the trabecular meshwork [60]. In addition, small molecules can be eliminated from the aqueous humor through the blood flow of the iris–ciliary body.

Retinal penetration of nanoparticles and large molecules can be limited by the inner limiting membrane (ILM) (Fig. 1). The pore size of the ILM is not clear, but some estimates are in the range of 100 nm, indicating that it is much leakier than the RPE (Table 1).

Anti-VEGF biologics are injected at intervals of 1–2 months and have intravitreal half-lives in the range of a week. These drugs are large molecules that permeate poorly across the blood–retinal barrier and, therefore, ~90% of the dose is eliminated via the anterior route [61]. Small-molecule drugs, capable of permeating across the blood–retinal barrier, have half-lives of 2–10 h in the vitreous, which would necessitate frequent intravitreal injections, unless slow drug dissolution (e.g., triamcinolone acetonide suspension) or release (e.g., Ozurdex implant) are achieved [60]. Retinal diseases require chronic treatments and the dosing interval is an important issue. Therefore, effective, less invasive, and long-acting drugs are needed.

## Dosing considerations

Drug bioavailability is considered to be complete after intravitreal injection (F = 1.0). Intravitreal delivery systems must be loaded with an adequate drug dose to enable prolonged duration of action (preferably 3–12 months). Obviously, longer dosing intervals ($\tau$), high vitreal clearance ($CL_V$),and low drug potency (high $C_{ss,av}$) lead to increased drug loading requirements in the formulation. Vitreal drug clearance in rabbits is well known; 0.05–1 ml/h for small molecules and 0.01–0.07 ml/h for protein drugs [58].

Equation (1) was applied to intravitreal drug delivery to estimate the required intravitreal drug doses ($D_V$) for various dosing intervals ($\tau$) and target concentrations ($C_{ss,av}$) in the vitreous during chronic treatment. We can calculate the required drug doses for 3 and 12 month-dosing intervals with target drug concentrations of 100 μM and 1 μM in the vitreous. For a small molecule (vitreal drug clearance $CL_V$ = 0.283 ml/h; assumed molecular weight of 500), the required drug doses for 3 and 12 months were determined. For $C_{ss,av}$ of 100 μM concentration, doses of 30 mg and 120 mg are needed for 3 and 12 months, respectively (Fig. 2C). The doses for a target concentration of 10 μM are 3 mg and 12 mg; for 1 μM concentration 305 μg and 1.2 mg; and for 0.1 μM 30 μg and 120 μg (Fig. 2C). For a protein drug (values of bevacizumab: $CL_V$ = 0.017 ml/h; molecular weight 149 kDa) the required drug doses were calculated for 3 and 12 months as follows: for a 10 μM target concentration, 55 mg and 220 mg; for 1 μM concentration, 5.5 mg and 22 mg; for 0.1 μM concentration, 0.55 mg and 2.2 mg; and for 10 nM concentration, 55 μg and 220 μg.

The maximum volumes for intravitreal injections are 50 and 100 μl in rabbits and humans, respectively. Larger injection volumes would increase the intraocular pressure, thereby causing ocular damage. The clinically used intravitreal implants have even lower volumes, ranging from 1.5 μl to 30 μl: Ozurdex (rod shaped, diameter 0.46 mm, length 6 mm, volume 3.9 $mm^3$), Retisert (3 mm × 2 mm × 5 mm = 30 μl), and Iluvien (cylindrical tube with length of 3.5 mm and width of 0.37 mm, volume 1.5 μl) [62]. Therefore, it is obvious that drugs used in long-acting intravitreal delivery systems should have target concentrations of <1 μM for small-molecule drugs and <0.1 μM for large molecules (Fig. 2C). Otherwise, the drug concentration in the device is too high for successful formulation of reproducible and stable devices. The conclusions for the human eye are expected to be similar, because the intravitreal clearance in the human eye is modestly (1.4 times) higher than in the rabbit eye [62].

Table 3 includes examples of intravitreal drug delivery systems that have been tested *in vivo*. We can compare the real intravitreal free drug concentrations and kinetic calculations (Eq. (1), Fig. 2c)

**TABLE 3**

**Examples of intravitreal drug delivery systems**

| Material | Drug or label compound | Drug loading (μg/mg) or total dose (μg) | Release time *in vitro* (days) or release rate [μg\|d] | Retention in vitreous/duration of action (d) | Concentration range (μM) in vitreous | Refs |
|---|---|---|---|---|---|---|
| **Liquid formulations** | | | | | | |
| PLGA nano/microparticles | Bevacizumab | 12.5 mg/ml of suspension; 62.5 μg | | >30 | 21–31 | [92] |
| PLGA nano/microparticles | Dexamethasone | 40 μg/mg; 200 μg | | >20 | 63 | [93] |
| **Semi-solid formulations** | | | | | | |
| PLGA-PEG-PLGA gel | Dexamethasone | 1 mg/ml | >10 days | >9 | 3–23 | [94] |
| **Solid implants** | | | | | | |
| Chitosan coated with PLGA | Methotrexate | 400 μg/mg; 400 μg | | 33 | 0.1–1.0 | [95] |
| PVA-silicone laminate (Retisert) | Fluocinolone acetonide | 590 μg | 0.3–0.6 μg/d | 2.5–3 years | 0.2–0.4 | [96] |
| PLGA (Ozurdex) | Dexamethasone | 700 μg | 1 μg/h | 90 | 0.6 | [97,98] |
| PVA, ethylene vinyl acetate (Vitrasert) | Ganciclovir | 4.5 mg | 1.4 μg/h | 150–240 | 7.5–29 | [99] |
| Silicone, polyvinyl alcohol (Iluvien) | Fluocinolone acetonide | 190 μg | 0.2 μg/day | 728 | 0.1–2 | [100] |

of commercially available implants that release dexamethasone (Ozurdex), fluocinolone acetonide (Retisert), and ganciclovir (Vitrasert) (Table 3). The simple calculations with Equation 1 match the real data well. In the case of Ozurdex, a target concentration of 0.6 μM should be maintained for 3 months with a drug dose of 183 μg (the device hosts 700 μg). Similar calculations for Vitrasert give 10.7 mg for 7 months' delivery (the device holds 4.5 mg). Retisert holds 590 μg of fluocinolone acetonide, whereas the kinetic calculations suggest that, to reach the target concentration of 0.2–0.4 μM, the device should contain 619–1238 μg of the drug. These comparisons demonstrate that Eq. (1) and Fig. 2C can be used to obtain reliable estimates of the required drug loading at the beginning of formulation development.

These calculations are based on the assumed retention of the device in the vitreous and drug release from the device in the vitreous. The concentrations refer to the free, pharmacologically active, drug concentrations in the vitreous, not the total concentrations. In Eq. (1), clearance is for the released and free drug and, therefore, the concentrations must also be for the free drug. The situation of dispersed and gel systems is often misleading, because total concentrations have been measured in such cases (Table 3).

Overall, it is clear that drug potency has a big impact on the feasibility of a compound for long-acting intravitreal systems (Fig. 2c,d).

## Drug delivery system design

The vitreous humor represents a unique environment for injected drug formulations: an isotonic clear gel (water content is 98–99%, viscosity of 300–2000 cP) occupies the vitreal cavity (Fig. 1). The vitreous humor contains collagen (mostly type 2), hyaluronic acid (HA), proteoglycans, and some hyalocyte cells. Collagen provides the solid structure to the vitreous, whereas HA yields the swelling pressure that spaces the collagen fibrils apart from each other [64]. The protein concentration in the vitreous is low and drugs are only modestly bound in the vitreous [65]. At the normal vitreal pH of 7.0–7.4, the vitreous humor bears a negative net charge that stems from HA and proteoglycans with carboxylic acid and sulfate moieties. Polylactide-based polymers can cause local acidification during degradation, potentially leading to adverse reactions [66]; nonetheless, the polylactide-based implant Ozurdex has been accepted for clinical use. It is possible that including the anti-inflammatory dexamethasone in the implant can help to alleviate the situation.

The vitreous humor has local differences: the central vitreous is more liquid than the peripheral parts and its viscosity is lower in older patients [67,68]. The posterior vitreous cortex comprises densely packed collagen fibrils arranged upon the ILM [68]. Diffusion studies indicate that the mesh size in the vitreous is ~550 nm [28], suggesting that even nonliquefied vitreous allows diffusion of drugs and nanoparticles, except positive nanoparticles, which bind to the vitreous humor [63].

Several types of drug formulation have been used intravitreally: gels, particles, and implants (Table 3). Drug delivery systems are in contact with the vitreous humor and release drug that will distribute to the target tissues, such as retina and choroid. Alternatively, the nanosized delivery system could distribute and deliver the drug to the target cells. The injected material should be endotoxin free, sterile, should not change pH, osmotic pressure or transparency of the vitreous, and should not induce the aggregation of the vitreous humor components.

Clinically used intravitreal drug products are simple solutions (e.g., ranibizumab) or implants. Nondegradable solid implants (e.g., Iluvien or Retisert) must be removed from the eye surgically or left in the eye as empty ghost matrices, whereas degradation products from biodegradable polymeric implants (e.g., Ozurdex) are eliminated from the eye. Water-soluble, but nondegradable polymers (e.g. polyethylene glycol or polyvinyl alcohol), are probably cleared from the eye primarily via the anterior route, such as FITC-dextran [69]. Unfortunately, polymer degradation and elimination in the vitreous have been rarely studied [63]. Degradation to smaller fragments should facilitate elimination from the eye [63], because smaller molecules are more rapidly eliminated, possibly also via the posterior route. Enzymes might contribute to polymer degradation, but there is only sparse information about the enzyme activity in the vitreous [63]. Metalloproteinases and their inhibitors are present in the vitreous (e.g., MMP-1, MMP-2, MMP-9, MMP-3, MMP-8, TIMP-1, TIMP-2, and TIMP-3) [70–72], as well as heparinase, cathepsin L, esterases [73], and peptidases [74]. Recently, some peptide linkers were shown to be cleaved fast in RPE cells and slowly (10% in 1 week) in the vitreous [74].

Gels can be used to sustain drug release in the vitreous, but they must be injectable through a small needle. *In situ* gel-forming materials are attractive, because they are nonviscous upon injection and form a gel after injection into the vitreous [75]. For example, thermosensitive hydrogel materials, such as PLGA-PEG-PLGA, will form a gel when the ambient temperature rises upon ocular injection [76,77]. Normally, diffusion of small drug molecules is relatively fast in the gel, limiting the duration of drug release; however, the duration of drug release can be extended by incorporating them into particles (e.g., polymeric micelles) within the gel [78]. Protein drugs diffuse slower in gels and longer durations of drug release can be achieved [77]. The application of HA derivatives as gelling material is an interesting option, because HA is a major component in the vitreous [77]. Cationic particles and gels should not be used, because they aggregate with HA within the vitreous.

Both microparticles and nanoparticles have been tested in intravitreal injections [79,80]. The size of injected particles is important, because greater particle size can lead to light scattering and potential vision problems, whereas nanoparticles generally do not affect the vision [81]. However, microparticles might have more extended drug release times than nanoparticles, whereas the latter can be also used for retinal penetration [79]. The size of the particles also affects their diffusion in the vitreous and retina. Importantly, the vitreous allows diffusion of larger particles than the ILM, which has a smaller mesh size (Table 1). If free drug (e.g., transcription factors or various forms of RNA) is not capable of reaching its intracellular targets, drug delivery systems are needed to transport and release them into the retinal target cells. Obviously, such carriers must diffuse through the vitreous humor and ILM. Even though diffusion of compounds and nanoparticles in the vitreous is slower than in water, the differences are not large, because the mesh size in the vitreous is 550 nm (Table 1) [28]. Anionic and neutral nanostructures (e.g., 100 nm in diameter) diffuse well in the vitreous, but cationic particles bind to HA and diffuse ~100–1000 times more slowly in the vitreous than in water

[63]. Polycationic materials can bind and even aggregate with the HA network in the vitreous, potentially inducing macrophage reaction. Aggregation can be reduced with PEGylation of polycations [82]; a procedure that increases particle mobility in the vitreous humor [83]. In addition to diffusion, there is also a slow convective flow in the vitreous towards the retina [84]. Vitreal liquefaction can change the time course of drug distribution in the vitreous, but its pharmacological importance is not certain [63,85].

For retinal delivery, the carrier needs to permeate across the ILM at the vitreous–retina interface (Fig. 1). The ILM has a smaller mesh size than the vitreous (Table 1), but the physicochemical determinants of molecular and particle permeation through ILM into the retina remain unclear. The ILM does not hinder permeation of soluble small molecules, oligonucleotides, or FITC-dextrans [86]. In the case of anionic and neutral nanoparticles, both penetration [79,87,88] and lack of retinal penetration [86,89,90] have been reported for particles with mean sizes of 132–350 nm. Interpretation of the data from a physicochemical viewpoint is difficult, because nanoparticles are not monodispersed, but instead have a size distribution. Possibly only the smallest particles in the injected sample enter the retina. The most consistent permeation is seen with soluble molecules, suggesting that neutral and soluble polymer conjugates or small nanostructures, such as PCL-PEG micelles [91], are ideal materials for drug targeting into the retina. The last phases in intracellular drug delivery (internalization, intracellular distribution, and release) are discussed elsewhere [63]. The intracellular delivery properties might be different for various retinal cell types.

## Subconjunctival injection

### *Drug targets and pharmacokinetics*

The conjunctiva is a thin, transparent mucous membrane, comprising an epithelium and stromal layers. The conjunctiva covers the anterior sclera (bulbar conjunctiva) and lines the inner side of the eyelids (palpebral conjunctiva) (Fig. 1). Subconjunctival injections are placed between the bulbar conjunctiva and sclera. They are used in the clinical practice to deliver drugs (e.g., local anesthetics and anti-inflammatory drugs) to the anterior segment of the eye. Experimentally, the subconjunctival route has been tested for anterior segment delivery of glaucoma drugs and posterior segment drug delivery of various compounds [63]. Current formulations for subconjunctival drug delivery are simple solutions or suspensions. The injected volumes are variable, typically 0.1–0.5 ml.

After subconjunctival administration, part of the injected solution might spill out to the tear fluid and, thereafter, be partly absorbed through the cornea into the eye [101]. However, the largest portion of a subconjunctivally injected drug will absorb to the lymphatic and blood circulation, which limits its ocular bioavailability [4,102,103]. Part of the injected drug distributes from the subconjunctival space across the sclera deeper into the eye [101]. The sclera is a relatively leaky tissue that allows diffusion of even macromolecules (Table 1) towards the iris and ciliary body. The bioavailability of subconjunctival small-molecule drugs in the aqueous humor is <0.1, but is generally higher than after topical administration. After subconjunctival injection, the drug can reach the iris and ciliary body directly from the sclera, without distributing via the aqueous humor.

Posteriorly, the drug permeates through the sclera to the choroid, RPE, retina, and vitreous [104]. The retinal bioavailability ($F_R$) is estimated to be only 0.0003–0.003 due to: (i) drug loss at the site of injection; (ii) scleral barrier; (iii) drug loss to the choroidal blood flow; and (iv) the RPE barrier (Table 1, Fig. 1) [104]. Choroidal bioavailability is higher (a few percent), but the drug concentrations in the choroid can be low because of the high clearance in this tissue [104,105]. Based on these pharmacokinetics, it is evident why subconjunctival delivery is currently used clinically only in the treatment of anterior segment disorders.

### *Dosing considerations*

The average steady-state free drug concentrations of a subconjunctival drug in the aqueous humor are usually in the same order of magnitude of those after topical drug delivery at equivalent dosing rate. Thus, Eq. (1) and Fig. 2A are useful for the early dosing rate estimates for subconjunctival administration. Let us consider latanoprost, the drug of choice in open-angle glaucoma. The ocular absorption of latanoprost from eye-drops (typical clinical dose of 1.5 μg) was determined in humans (patients undergoing cataract surgery) [106]. For 4 h post dosing, the drug concentrations in the aqueous humor were 3–32 ng/ml, whereas prediction with the standard values (see earlier) with Equation 1 yielded $C_{ss,av}$ of 12 ng/ml. Furthermore, a subconjunctival liposomal formulation reduced the intraocular pressure in patients for 90 days after single injection of latanoprost (dose = 100 μg), suggesting that the anterior segment bioavailability after subconjunctival injection is in the same range as topical bioavailability, because the average daily dose subconjunctivally was 1.1 μg [107]. Cheng *et al.* [108] developed a polymeric thermosensitive hydrogel (chitosan/gelatin/glycerol) that released latanoprost in a controlled rate. After subconjunctival injection of latanoprost (50 μg) in the gel to the rabbit eyes, the drug concentrations in the aqueous humor were 2.8–6.3 ng/ml, whereas the calculation with standard values for topical delivery (F = 0.02, $CL_{ah}$ = 10 μl/min) resulted in a $C_{ss,av}$ value of 2.6 ng/ml. Also, a single injection of liposomal latanoprost (dose = 100 μg) resulted in controlled drug release and reduction of intraocular pressure in rabbits [109] and monkeys [110] for 90 and 120 days, respectively. The calculations and experimental data match relatively well, suggesting that Eq. (1) and Fig. 2A are useful aids in the evaluation of the required dosing rates for subconjunctival drugs with anterior chamber targets in rabbits and humans.

Subconjunctival delivery results in low bioavailability ($F \approx 0.001$) in the neural retina and vitreous [104]. Figure 2b shows the relationship between drug dose and dosing frequency for drugs with different target concentrations in the retina (F = 0.001, CL = 0.283 ml/h). It is evident that this mode of drug delivery might be suitable only for very potent drugs with required $C_{ss,av}$ of 10 nM or less. For instance, a 1 μM retinal drug concentration can be maintained for 1 month with ~100 mg of small-molecule drug. The dose requirement for an equipotent protein drug would be even higher and subconjunctival delivery of biologics might be suitable only for drugs with target concentrations of 1 nM and below. Even though the subconjunctival space can accommodate larger delivery systems (e.g., 200 mg) than the vitreous, the applicability of this route is limited to highly potent retinal drugs.

### *Drug delivery system design*

Some researchers have proposed to use nanomedicines for targeted drug delivery from the subconjunctival space into the retina [111–113], but the tissue barriers might limit the access of the carriers to the retina. Based on the pore sizes of sclera and RPE, nanoparticles are not expected to be able to permeate to the retina. The studies in this field are based on qualitative imaging and it is unclear whether a pharmacologically significant permeation occurs in large eyes (e.g., in rabbits or human).

Many studies have presented controlled-release systems for subconjunctival drug delivery. For example, liposomes [107,109,110], thermosensitive hydrogels [108], and other polymeric controlled-release systems [114] have been developed. Such systems prolong drug retention at the subconjunctival site of injection. Elimination from the injection site is fast without a delivery system: for example, the amount of hydrophilic manganese ethylene diamine tetraacetic acid complex at the subconjunctival injection site decreased by 90% in 1 h [115,116]. Rapid removal of the drug to the blood and lymphatic circulation can be prevented with drug delivery systems, thereby providing effective means to prolonged drug action.

## General discussion

In the calculations presented earlier, we assumed that average steady-state concentrations are achieved in the pharmacokinetic sampling compartment (aqueous humor or vitreous). This is a relevant approach for multiple dosing regimens. At steady state, the free drug concentrations in the aqueous and vitreous humor compartments are at equilibrium with the surrounding target tissues (e.g., retina or trabecular meshwork). Our approach enables the use of simple calculations that are easily accessible to researchers and drug developers for assessing potential routes of ocular drug administration, drug payloads, and dosing intervals.

The required target concentration (i.e., $C_{ss,av}$) is a key parameter in Equation 1. For new compounds, the target concentration can be estimated based on *in vitro* assays, using $IC_{50}$, $K_i$, or $K_d$ values as starting points, but not using those values as such. Rather, 1–2 orders of magnitude higher concentrations are more relevant in ensuring adequate drug efficacy; assuming complete occupancy of the target protein. For known drugs, prior *in vivo* experiments are helpful in defining the target concentrations.

The estimates of drug bioavailability in the target area (F) and clearance at the target compartment (CL) are needed in Eq. (1). Bioavailability values are relatively constant in the case of intravitreal (1.0) and subconjunctival delivery (~0.1 for the anterior chamber and ~0.001 for the retina) regardless of the drug properties [60,104]. However, the bioavailability range for topical application is wider (~0.001–0.05). In that case, quantitative structure–property relationships (QSPR) for corneal and conjunctival permeability can be used to reach more accurate bioavailability predictions [21]. The clearance range in the anterior chamber is relatively narrow (less than an order of magnitude for small-molecular-weight drugs). The clearance values of small-molecule drugs in the vitreous span a 49-fold range (0.031–1.53 ml/h) [60]. Nevertheless, relatively accurate predictions for the clearance of individual compounds can be obtained with a simple QSPR model [60]. By contrast, the vitreal clearances of protein drugs are within a threefold range in the rabbit eye [60]. The clearance values can also be scaled up to the human eye (vitreal clearance in human patients is 1.4 times higher than from the rabbit vitreous) [63].

In the dosing estimates (Fig. 2), we assumed that the whole drug load is released at the administration site during the dosing interval and the delivery system is not eliminated from the site of application before drug release. Constant zero-order release will result in a constant steady-state concentration in the target compartment, but often drug release obeys first-order kinetics (constant fraction released at each time interval and the absolute release rate decreases with time). In that case, the $C_{ss,av}$ obeys Eq. (1), but the peak concentrations will be higher than $C_{ss,av}$ and the trough concentrations will be below $C_{ss,av}$. Pharmacological implications of this situation are case dependent (mechanism of drug action and disease state). In many cases, drug action can prevail even when the drug concentration falls below the threshold concentration during the multiple dosing regimen. In those cases, $C_{ss,av}$ can be used as a target, allowing concentration fluctuations above and below the $C_{ss,av}$ levels. Higher drug loading in the delivery system is needed if a first-order release system is used to maintain drug concentrations continuously above a certain target concentration [60]. For a full prediction of response versus time curves, pharmacokinetic/pharmacodynamic modeling can be used, but this approach requires expert skills; for an example, see pharmacokinetic/pharmacodynamic modeling of ranibizumab action on VEGF [114].

We used aqueous and vitreous humor as target compartments in the calculations, but the drug targets are usually located in neighboring tissues, such as iris, ciliary body, trabecular meshwork, and retina. At steady state, the concentrations of small-molecule drugs equilibrate with these tissues. This assumption is valid in most cases. However, some possible deviations are obvious: (i) large molecules with intracellular targets do not get access to their targets without special delivery systems; (ii) active influx or efflux of drugs in the target cells; and (iii) drug metabolism in the target tissues. Thus, this approach should not be used for large molecules with intracellular targets (e.g., siRNA or transcription factors). The role of active transport and metabolism in the ocular tissues is unclear and there are no known cases where these processes would dominate ocular kinetics [19].

## Concluding remarks

Ocular drug delivery is a complex field in which multiple factors determine the drug concentrations and, thus, efficacy at the target site. Furthermore, drug loading and required release rates are strongly dependent on the local site of drug administration and location of the target tissue. Here, we have presented a simple guideline for dose and delivery system selection to augment decision-making and initial drug delivery system design in ophthalmic drug development.

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