ELSEVIER

Ocular biopharmaceutics: impact of modeling and simulation on topical ophthalmic formulation development

Hovhannes J. Gukasyan, Shumet Hailu, Thomas K. Karami and Richard Graham

Early Pharmaceutical Development, Allergan plc, 2525 Dupont Drive, Irvine, CA, 92612, United States

The estimation of ocular pharmacokinetics (PK) in various eye tissues is limited because of sampling challenges. Computational modeling and simulation (M&S) tools underpinning the elucidation of drug access routes and prediction of ocular exposure are essential for the mechanistic assessment of biopharmaceutics in the eye. Therefore, theoretical and experimental evaluation of ocular absorption and transit models is necessary. Biopharmaceutical parameter sensitivity analysis based on permeability and drug dose illustrates utility in ocular drug delivery assessment, which could have innovative and cost-saving impacts on ophthalmic product development and therapeutic bioequivalence (BE) evaluations.

Introduction

Topical ophthalmic dosage forms are the historically preferred administration means to treat a variety of ocular surface and anterior segment disorders [1]. The US Food and Drug Administration (FDA) *Orange Book* lists 591 product entries under topical ophthalmic route of administration, with 106 active pharmaceutical ingredients that can be identified within this subset, suggesting at least a 1:5 drug to formulation ratio [2]. This highlights the prevalence of topical ophthalmic product reformulations containing identical active ingredients. Given sampling challenges associated with ocular PK to establish BE between different formulations, the need for predictive mechanistic modeling is obvious, whether the goal is to develop a new chemical entity or reformulate an existing one for ophthalmic indications [3].

In recent years, i\njectable liquid dosage forms and implantable drug delivery devices have been developed to specifically address ocular PK challenges associated with retinal diseases, because the topical route remains inadequate for the delivery of drugs to the posterior segment. Intraocular injections are the only way to guarantee complete and accurate dose delivery to treat back of the eye disorders [4]. Anatomical barriers presented by the ocular surface, limited space for local drug delivery, unique patient demographics (especially in glaucoma [5]), and the immune-privileged nature of the eye necessitate ophthalmic dosage forms to be highly specialized parenterals. Topical ophthalmic dosage forms are additionally burdened with low ocular bioavailability (e.g., only a fraction of the dose absorbed into the eye from an instilled drop) by virtue of corneal and conjunctival epithelial barriers, blink reflex, tear production, and nasal drainage responsible for the rapid turnover of tear film resident over the ocular surface [6].

The primary and most direct route of drug penetration into anterior intraocular compartments occurs via transcorneal mass transfer. Corneal tissue comprises only ~20% of the ocular surface and presents 'tight' lipophilic barrier properties [6]. A secondary route by which molecules can reach front segment intraocular tissues is the transconjunctival pathway, which has inverse properties to the cornea [7], such as a 'leaky' barrier [8,9]. Although ~80% of the ocular surface is covered by the conjunctiva, it is a highly vascularized tissue partially with a role in undesirable systemic exposure from topical eye drops. Optimization strategies of transcorneal and transconjunctival mass transfer have been suggested in reports that methodically examined the fine-tuning of molecular, physicochemical, and formulation parameters (i.e., osmolarity, pH, net charge, viscosity, shear-thinning, and local and general anesthesia) in topical ophthalmic drug products [10–15]. Physiologically based and anatomically accurate, (global) computational

Corresponding author: Gukasyan, H.J. (hovhannes.gukasyan@allergan.com)

https://doi.org/10.1016/j.drudis.2019.04.002

models of the eye have been designed, developed, and commercialized in recent years, which is significant and impactful considering the aforementioned empirical challenges with ocular drugs and drug delivery. In this review, we discuss known, robust, and reliable biopharmaceutical models that demonstrate the impact of absorption and formulation parameter sensitivity towards ocular bioavailability, intensity, and extent of exposure. We also provide a brief chronological overview on reports describing ocular biopharmaceutics.

Historical perspective

David M. Maurice published on corneal physiology in a 1953 review discussing drug penetration into the eye from drops instilled within the conjunctival cul-de-sac and the limiting role of ocular epithelial membranes, chronologically, dating back to 1853, referencing various publications on 'Test Substance' permeation across the cornea [16]. Wine *et al.* in 1964 and McCartney *et al.* in 1965 reported the earliest plausible studies using a systematic approach examining mechanisms of hydrocortisone penetration into the eye following subconjunctival injection; notably from the perspective of health versus disease, time-dependent PK, and routes of absorption [17,18]. To our knowledge, these are likely the two earliest reports in which the corneal route of permeation was emphasized by observations suggesting that subconjunctivally injected hydrocortisone partially reaches efficacious intraocular levels by first refluxing out of the injection site, and then mixing with the tear film, to finally re-enter the eye transcorneally [17] (a noteworthy side note and segue to pilocarpine absorption, Conrad and Robinson reported the same penetration mechanism following subconjunctival injection [19]). Subsequently in 1978, Doane *et al.* recapitulated the preferred mechanism of transcorneal absorption of hydrocortisone using a cylindrical well over the corneoconjunctival limbus to allow for controlled topical drug solution access to the cornea or conjunctiva only. Moreover, their interest in ocular inserts at the time led them to investigate pilocarpine hydrochloride using the same model system [20]. This study was a turning point in early topical ophthalmic biopharmaceutics because it demonstrated differences in the barrier roles of the cornea and conjunctiva for a hydrophobic and hydrophilic drug (hydrocortisone and pilocarpine, respectively) reaching peak concentrations in the aqueous humor or iris–ciliary body (ICB). It became clear from these results that the distribution of a topically applied drug in the tear film has a key role towards subsequent entry into anterior segment compartments, leading to the complete mechanistic mathematical model for pilocarpine disposition reported by Lee and Robinson in 1979. Individual influences of precorneal absorption and loss parameters, including formulation effects, on the ocular bioavailability of pilocarpine were assessed deriving predictive, although specific to pilocarpine, precorneal and anterior intraocular models from experimental findings [21]. By using a physiologically based ocular PK (PBPK) model, Miller *et al.* were able to rationalize several studies of topically applied pilocarpine eye drops in an extrapolative manner, showing good agreement between predictions and measurements of the concentration over time profile of the drug [22]. Next, in 1985, the Doane *et al.* 'cylindrical cup' model was adapted by Ahmed and Patton to investigate routes of topical ophthalmic drug absorption with a pair of different probe molecules, timolol and inulin. Primarily based on intraocular exposure results from inulin eye drops (a ~6.2 kDa fructosan), these studies demonstrated several key biopharmaceutical considerations: (i) absorption by the 'noncorneal route' (e.g., conjunctiva–sclera pathway) allows drug entry into ICB bypassing the cornea and aqueous humor; (ii) the same pathway is significant for drugs that are poorly absorbed across the cornea; and (iii) the physicochemical and formulation properties of the drug influence mass transfer via a pair of available absorption routes (e.g., transcorneal or transconjunctival) from topical ophthalmic dosage forms instilled in precorneal space [7]. Lee and Robinson, in their 1986 review, provided guidance stressing the importance of ocular PK modeling, bioavailability studies, and choice of animal models in the design and biopharmaceutical evaluation of progressive topical ocular drug delivery systems. Since then, much progress has been made around concepts such as the posterior segment delivery of therapeutics from topical dosing and ocular delivery of macromolecules derived from biotechnology; however, ultimate translation to humans has yet to be demonstrated [6,23]. Here, we provide an analysis of current progressive computational M&S approaches (with an exemplification) guided by a brief overview of groundwork in ocular PK and biopharmaceutics research generated over the past four decades.

Modeling ocular biopharmaceutics

The application of biopharmaceutical assessments, including M&S, to support drug discovery and development has gained increasing industry and regulatory acceptance, mainly for oral drug delivery [24–26]. In general, the M&S approach has significantly advanced the rational design of drug molecules based on quantitative structure–activity and –property relationships (QSAR/QSPR) and has guided drug product development to improve *in vivo* performance. Additional applications of M&S in drug development include human PK prediction from *in vitro* and *in vivo* animal data, supporting dose selection and clinical protocol design, as well as conducting BE assessments for biowaivers to minimize the need for expensive relative bioavailability (rBA) or BE studies [25]. Even though there has been some success in biopharmaceutical modeling of oral drug delivery, extension of this practice to topical ocular and other absorption-dependent drug delivery systems (e.g., the topical classification system proposed by Shah *et al.* [27]) is still underway.

Detailed reviews with tabulated hyperlinks of M&S platforms are available elsewhere [24,26]. Some of these biopharmaceutical modeling programs (e.g., GastroPlus™ and Simcyp) have built-in modular equations derived from empirical training data sets, and can facilitate structure-based predictive modeling using physicochemical properties while requiring minimal experimental data. Other programs, such as MATLAB®, are flexible towards end-user customization and can be extended to obtain desired biopharmaceutical models adapted for almost any drug delivery system. Applications of ocular biopharmaceutical M&S to support ophthalmic drug discovery and development have gained significant momentum over the past decade. A few recently developed software programs showed promising results in applications of ocular biopharmaceutics assessment.

User-customizable programs [e.g., OCUSIM (Ocular Simulator) a MATLAB® platform based on physiological parameters of eye tissues (compartment sizes defined in terms of volume and surface area from several species) and user-defined inputs for physicochemical parameters of drugs] have been used to model ocular drug PK following topical instillation and intraocular injection of several proprietary compounds and triamcinolone acetonide (TA), respectively [28,29]. Following topical or sub-Tenon's capsule dosing, in rabbits or patients, respectively, ocular and systemic PK data were successfully predicted in aqueous humor, vitreous humor, ICB, and serum as total concentrations over time for several new chemical entities and TA [28,29]. OCUSIM provided an understanding of drug distribution and prediction of concentrations in various eye tissues that were otherwise difficult to measure experimentally. Lastly, mass transfer phenomena in aqueous and vitreous humor are mathematically described via diffusion, convective flow, or finite element modeling [30–33]. Missel *et al.* reported a computational fluid dynamics simulation of vitreous humor, specifically to study dissolution of intravitreally injected TA suspensions. Among several crucial attributes, this anatomically accurate intravitreal suspension formulation dissolution model in rabbit suggested that the duration of PK from intraocularly injected TA depots would be independent of particle size [33].

The Ocular Compartmental Absorption and Transit (OCAT™) software module (version 3) in GastroPlus™ (Simulations Plus, Inc.) is the most advanced commercially available program that supports mechanistic ocular absorption modeling. OCAT™ not only has default built-in ocular physiologies for humans, rabbits, and monkeys, but also provides the option to customize or create a user-defined ocular physiology architecture. Various eye compartments considered in OCAT™ modeling are precornea, corneal epithelium, corneal stroma, bulbar conjunctiva, palpebral conjunctiva, aqueous humor, anterior sclera, posterior sclera, ICB, choroid and retinal pigment epithelium (RPE), retina, and anterior and posterior vitreous humor. Systemic drug absorption from drainage through nasolacrimal duct and from vascularized eye tissues (e.g., conjunctiva, ICB, choroid–RPE, and retina) into general circulation is also included. The PBPK model in GastroPlus™ is used to calculate the distribution of drugs reaching systemic circulation. Drug absorption and transport in various eye compartments is described by passive diffusion (calculated using permeability, surface area, and drug concentration in each tissue), carrier-mediated processes (using total expression level of transporters in relevant tissues), and convective flows. Drug metabolism and melanin binding in certain tissues can be modeled using linear or saturable processes. Total enzyme expression level and melanin content in relevant eye tissues are used in determining the metabolism and extent of melanin binding of the drug, respectively.

Ocular tissue mass transfer

Drug permeability between various eye compartments is a major factor influencing ocular absorption and distribution. Hence, apparent or effective permeability constants (P_{app} or P_{eff}) are key input parameters in ocular biopharmaceutical M&S to support drug discovery and development efforts. In OCAT™ modeling, ocular permeability is calculated from molecular structure, physicochemical, and biopharmaceutical properties of compounds using built-in QSAR predictive models (ADMET™ Predictor where applicable in GastroPlus™). Alternately, the same can be calculated from empirical models for various eye tissues that serve as major barriers for absorption (or elimination into systemic compartment) [1]. Here, we briefly review theoretical and experimental permeability models reported in literature for relevant eye tissues as a proxy to building a global computational ocular biopharmaceutical model.

Corneal permeability

Major corneal tissue layers that present barrier properties to mass transport are the outer epithelium, middle stroma, and inner endothelium. Extensive reviews of ocular permeability [34] and derivation of theoretical models [35,36] to predict corneal permeability as a function of molecular size and lipophilicity are available in the literature. An inverse relationship of molecular size and permeability has been shown for epithelium, stroma, and endothelium. Corneal epithelial and endothelial permeabilities increased with gained drug lipophilicity, whereas stromal permeability showed no clear dependence on distribution coefficient (log D) [35,36]. Kidron *et al.* [37] used multivariate computational analysis to derive a model with log D and hydrogen bond formation tendency as descriptors of overall corneal permeability. Kidron *et al.*'s model correlated well with experimental corneal permeability of compounds and was able to predict drug concentrations in aqueous humor. Fu *et al.* [38] reported another corneal permeability prediction model using three molecular parameters: net atomic charges of oxygen and nitrogen, net atomic charges of hydrogen atoms attached to oxygen and nitrogen, and molecular volume [e.g., V (A^3) using atomic radii to calculate polar molecular surface areas and molecular volumes by method of Clark [39,40]]. Fu *et al.*'s model explains the lower observed corneal permeability of compounds with higher net atomic charge by the tendency to form intermolecular hydrogen bonds. The lower permeability of ionized molecules is expected, assuming only neutral species partition into the phospholipid bilayer. The negative impact of molecular weight on corneal permeability was demonstrated using probe compounds with extended molecular size range [38] and is consistent with several other reports [34–36]. Lower diffusion coefficients through theoretical equivalent water filled pores in the cornea could result from excluding the mass transfer of larger molecules.

Drug absorption across the lipophilic layers of corneal epithelial multilayer and endothelial monolayer cells involves both paracellular and transcellular pathways. By contrast, mainly a paracellular diffusion pathway is available across the aqueous corneal stroma fibrous tissue. Therefore, various theoretical and experimental models converge on a consensus that epithelium is rate-limiting for corneal permeability of hydrophilic compounds, whereas stromal and endothelial permeabilities are important for lipophilic compounds [37,38,41–43]. Thus, for optimal permeability through different layers of corneal tissue, an adequate hydrophilic–lipophilic balance is essential.

Conjunctival permeability

An equation derived by Wang *et al.* [9] reported increased conjunctival permeability with lipophilicity having a sigmoidal relationship (as opposed to parabolic). The correlation was developed from similar molecular weight (typically 250–320 Da) β-blocker permeability values exhibiting various lipophilicities measured in

excised pigmented rabbit conjunctivas. Permeability was found to be more sensitive in the log P range of 1–3, qualitatively similar to that of corneal tissues. However, the absolute value of conjunctival permeability was higher and less sensitive to log P than its corneal analog (e.g., ~2–25-fold higher permeability depending on the log P of the compound). These observations were independently confirmed by another study [34], suggesting that the ratio of corneal to conjunctival permeability ($^{cr}P_{app}$:$^{cj}P_{app}$; a concept further discussed in exemplification section of this review) is relatively independent of changes outside of the log P 1–3 range [9]. Ramsay *et al.* reported a negative impact of molecular polar surface area and hydrogen bond donors on both conjunctival and corneal permeability in porcine tissues. Furthermore, a positive correlation was observed with the halogen ratio of the molecules [i.e., Σ(F, Cl, Br, I) ÷ Σ heavy atoms (excluding hydrogen)] [44,45]. A quantitative comparison based on the permeability data of 25 drugs showed that porcine conjunctival permeability was approximately ninefold higher than porcine corneal permeability. A chemical structure based ($N = 32$) *in silico* permeability model was reported for the first time, with eight in-common examples from Table 1, in porcine conjunctival tissues [45]. This could explain the presumption that the conjunctiva is the likely route for ocular absorption of large and/or hydrophilic molecules. Careful examination of ophthalmic biopharmaceutics literature reveals a gross bias of unavailable conjunctival permeability data for topical ophthalmic drugs with known corneal permeability [34,44], largely rendering the importance of transconjunctival ocular drug absorption suppositional [46]. Furthermore, because the conjunctiva is highly vascularized, with a partial role in systemic exposure following absorption of drugs from tears [6,46], examples reviewed here simultaneously model systemic and ocular absorption across palpebral and bulbar conjunctiva, respectively, using OCAT™ human version 3 'SplitFrontEye' (Simulations-Plus, Lancaster CA) model.

Scleral permeability

A theoretical model derived by Edwards *et al.* [36] showed a negative dependence of scleral permeability on molecular size. Similar to the corneal stroma, sclera is a fibrous tissue and, therefore, models developed to predict corneal stromal permeability can be applied for the approximate prediction of scleral permeability. In their review, Prausnitz and Noonan discussed a similar trend of decreasing scleral and corneal stromal permeabilities with increasing molecular size [34]. Extensive investigation of transscleral drug delivery as an alternative to ocular injections for posterior segment pharmacotherapy has been conducted, considering QSAR/QSPR for both passive-diffusive and electrotransport and/or electro-osmosis-facilitated mechanisms [47]. Overall, scleral permeability is higher than that of the cornea, which is reflected by the large amount of laboratory research and clinical interest in drug delivery to the posterior segment of the eye utilizing the trans-scleral route [48].

Choroid–RPE permeability

The relation of choroid–RPE permeability with molecular weight and lipophilicity of compounds was evaluated by Pitkänen *et al.* [49]. Probe hydrophilic molecules having a wide range of molecular weights and β-blockers with different lipophilicity (but narrow molecular weight range ~250–320 Da) were used to measure permeability coefficients in isolated bovine tissues. Choroid–RPE permeability increased with increasing lipophilicity of β-blockers but decreased with increasing molecular size for the hydrophilic probes. The increase in choroid–RPE permeability with lipophilicity is a similar observation to that reported for conjunctiva and cornea [9,34–37]. In addition, a decrease in permeability with molecular size was also observed for sclera and cornea [34,36,38]. Comparison of choroid–RPE to scleral permeability indicated the same range of values for the sensitivity of lipophilic molecules [49]. However, choroid–RPE permeability was significantly lower than scleral permeability for hydrophilic compounds and macromolecules, suggesting that this tissue has a major barrier role depending on the route of administration and type of molecule.

ICB permeability and aqueous/vitreous humor

Mass transfer M&S to and from the ICB is more abstract, because the ICB is often a target tissue for drugs (e.g., pharmacological modulators of aqueous humor production), and a reservoir of pigment/melanin that serves as a depot by reversible binding of drug molecules. ICB is similar in terms of barrier properties to the choroid–RPE [50]. Hence, within the context of ocular M&S, the same predicted permeability values can be applied to both tissues. Additionally, from a topical ophthalmic delivery perspective, the models consider mass transfer phenomena from aqueous humor and anterior sclera direction into ICB. M&S programs, such as OCAT™, can approximate the distribution and exchange of drug molecules within humor-containing compartments utilizing estimated or calculated aqueous diffusion coefficients.

Overall, modeling ocular permeability either using built-in QSAR prediction (e.g., ADMET™ Predictor in GastroPlus™ for OCAT™) or theoretical approaches reported in the literature for various eye tissues adds significant capability to ophthalmic biopharmaceutics in drug discovery and development. Prediction of permeability and drug distribution in hard-to-sample ocular compartments can be achieved using physicochemical, biopharmaceutical, and structural properties and validated against representative experimental data sets for novel compounds. Applications of ocular biopharmaceutical modeling could have an increasingly impactful role in novel ocular drug delivery systems, in addition to new molecular entity discovery, where QSAR/QSPR can be applied towards rational drug design [51].

OCAT™ exemplification of ophthalmic biopharmaceutics

Using OCAT™ version 3, a diverse set of 22 existing ophthalmic drugs (Table 1) was selected to build a confirmatory *in silico* model illustrating two overarching biopharmaceutical concepts: (i) mechanisms of drug entry into anterior segment tissues from an instilled topical eye drop formulation; and (ii) impact of hypothetical formulations on surrogate intraocular tissue exposure, as exemplified in Figs. 1 and 2 [52], respectively. ADMET Predictor was used to import chemical structures from Table 1 into GastroPlus™ and perform parameter sensitivity (PSA) analysis in OCAT™ on PK, permeability, and dose simulations illustrating the biopharmaceutical relationships governing absorption mechanisms and degree of bioavailability from topical ophthalmic route (Fig. 1) [52,53]. Apparent corneal and conjunctival epithelial tissue permeability (P_{app}) values were considered in the proposed ocular biopharmaceutical

TABLE 1

Topical ophthalmic drug substances with reported tissue permeability, intrinsic solubility, and product dose concentration utilized the proposed ocular biopharmaceutical classification system (oBCS)[a]

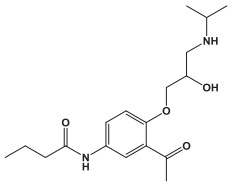
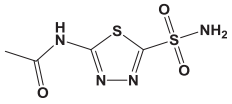
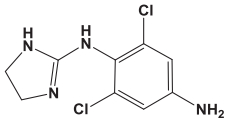
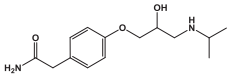
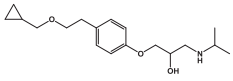
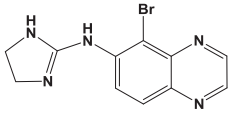
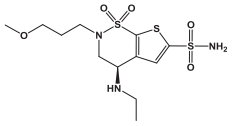
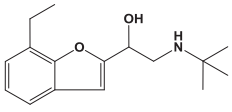
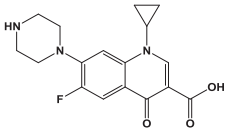
	Chemical structure, name	Apparent permeability conjunctiva; cornea ($^{cj}P_{app}$ and $^{cr}P_{app} \times 10^{-6}$ cm/s)	Solubility[e]; formulation concentration (mg/ml)
1	 Acebutolol	3.24 [9,34,52,53]; 3.62 [9,34,52,53,58,59]	0.558 [60]; 10 [58,61]
2		3.39 [52,53]; 1.28 [34,52,53]	0.7112 [62]; 10 [63]
3	Acetazolamide 	12.6 [52,53,64]; 3.65 [52,53,64]	0.409 [65]; 10 [66]
4	Apraclonidine 	4.95 [34,52,53]; 1.79 [34,52,53,59]	13.3 [67]; 10 [61]
5[b]	Atenolol 	5.24 [34,52,53]; 36.5 [34,52,53]	0.451 [68] or 0.03 [65]; 5 [68]
6	Betaxolol 	6.73 [52,53,64]; 28.8 [52,53,64]	29.85 [69]; 2 [66]
7[b]	Brimonidine 	5.15 [52,53]; 0.91 [52,53]	0.749 [70]; 5 or 10 [70]
8[b]	Brinzolamide 	3.58 [52,53]; 22.4 [34,52,53,58,59]	0.0356 [68] or 0.214 [71]; 10 [58]
9	Bufuralol 	4.84 [52,53]; 0.42 [52,53,72]	0.17 [73]; 3 [68]

TABLE 1 (*Continued*)

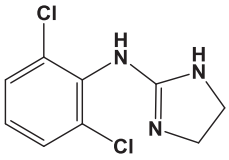
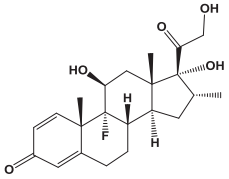
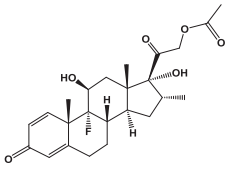
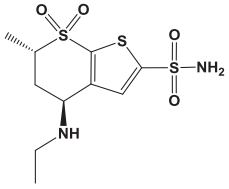
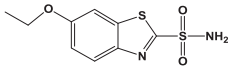
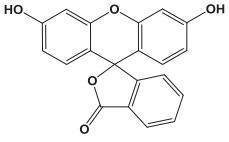
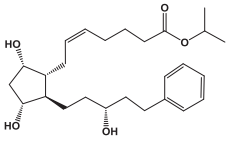
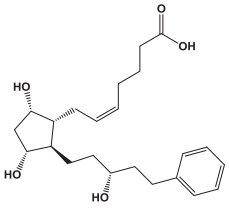
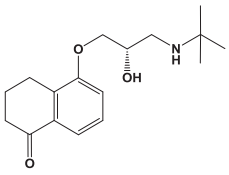
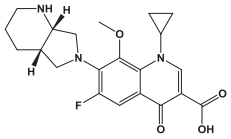
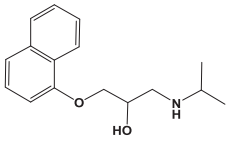
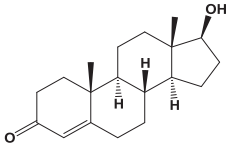
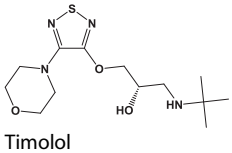
	Chemical structure, name	Apparent permeability conjunctiva; cornea ($^{cj}P_{app}$ and $^{cr}P_{app} \times 10^{-6}$ cm/s)	Solubility[e]; formulation concentration (mg/ml)
10[b]	Ciprofloxacin 	12.6 [52,53,64]; 46.7 [34,52,53,64]	13.6 [71] or 0.48 [65]; 1.25 [66]
11	Clonidine 	4.38 [52,53]; 5.08 [34,52,53]	0.089 [74]; 1[c] [75]
12	Dexamethasone 	5.44 [52,53]; 19.5 [34,52,53]	0.025 [71]; 1 [76]
13	Dexamethasone acetate 	4.17 [52,53]; 0.99 [52,53]	4.22 [77]; 20 [2]
14	Dorzolamide 	1.90 [52,53]; 25.9 [34,52,53]	0.04 [74]; 3 [63]
15	Ethoxzolamide 	3.84 [52,53]; 1.07 [34,52,53]	0.13 [78]; 100[d] [68]
16	Fluorescein 	4.77 [52,53]; 96.8 [52,53]	0.013 [68]; 0.05 [68]
17	Latanoprost 	2.59 [52,53]; 0.59 [52,53]	0.158 [79]; 0.055[c]

TABLE 1 (*Continued*)

Chemical structure, name		Apparent permeability conjunctiva; cornea ($^{cj}P_{app}$ and $^{cr}P_{app} \times 10^{-6}$ cm/s)	Solubility[e]; formulation concentration (mg/ml)
18	Latanoprost acid 	5.51 [9,34,52,53]; 19.5 [34,52,53,59]	0.25 [65,68,79]; 5 [65,68]
19	Levobunolol 	5.98 [52,53]; 8.91 [52,53,80]	0.17 [65,68,79]; 5 [80]
20	Moxifloxacin 	2.48 [9,34,52,53]; 38.0 [34,52,53,59]	0.062 [67]; 10 [81]
21	Propranolol 	2.20 [52,53]; 32.9 [34,52,53]	0.0234 [82]; 30 [83]
22[b]	Testosterone  Timolol	5.15 [9,34,52,53]; 18.9 [8,9,34,52,53,58,59]	0.27 [68,79]; 5 [7,8] or 10 [58,61]

[a] Adapted from [52,53].
[b] Betaxolol, brinzolamide, bufuralol, clonidine, and timolol have multiple reported solubility estimations or product dose concentrations. For multiple solubility reports, all permutations with reported dose concentration are incorporated within Fig. 1 (main text). For multiple dose concentrations, all product strength examples are incorporated within Fig. 1 (main text).
[c] Prodrug metabolites (e.g., dexamethasone from dexamethasone acetate and latanoprost acid from latanoprost). Metabolites are incorporated within Fig. 1 (main text) twofold; either as a stand-alone topical dose data point, or in terms of exposure data points resulting from their respective parent compound. Latanoprost acid shows a calculated molar equivalent dose concentration to parent prodrug.
[d] Fluorescein eye stain is a diagnostic test used to detect damage to the cornea or foreign bodies in the eye. Fluorescein is used within Fig. 1 (main text) as a model compound for its reported high dose concentration in certain commercial topical products.
[e] For ionizable species or zwitterions, solubility values of the neutral form are referenced for bases reported at a pH > 2 units above the pKa of the corresponding conjugate acid, for acids at a pH < 2 units below the pKa of the corresponding conjugate base, for zwitterions at a solubility versus pH minimum (isoelectric point).

classification system (^{o}BCS, Fig. 2) to explain target formulation concentrations required for solubilized doses (Table 1). Selected drug delivery dimensions in Fig. 1 are related to absorption from the precorneal space. Using the OCAT^{TM} v3-'SplitFrontEye' human model to replicate previously published experimental conditions of allowing access versus blocking precorneal-to-corneal mass transfer [7,20], all the compounds in Table 1 were subjected to PSA. Calculating ICB exposure at 20 min after topical solution dosing [Fig. 1, z-axis, ICB-AUC $_{0\text{-}20min}$(ng-h/ml) with or without corneal access] biopharmaceutical PSA was performed on a range of simulated doses and permeabilities for compounds in Table 1. OCAT^{TM} PSA varied the known therapeutic dose and estimated permeability for each drug (Fig. 1, x- and y-axis, respectively) orders of magnitude below and above baseline values, while fixing intrinsic aqueous solubility (Table 1 shows the reported baseline values for permeability and dose, and published intrinsic solubility) and dose volume (i.e., to 0.03 ml) [52,53]. The non-corneal mechanism of absorption was underscored in the whole-eye configuration, especially for compounds with low apparent corneal permeability (in good agreement with Table 1), where having complete formulation access to the corneal epithelium did not significantly change ICB exposure [7,20,52,53].

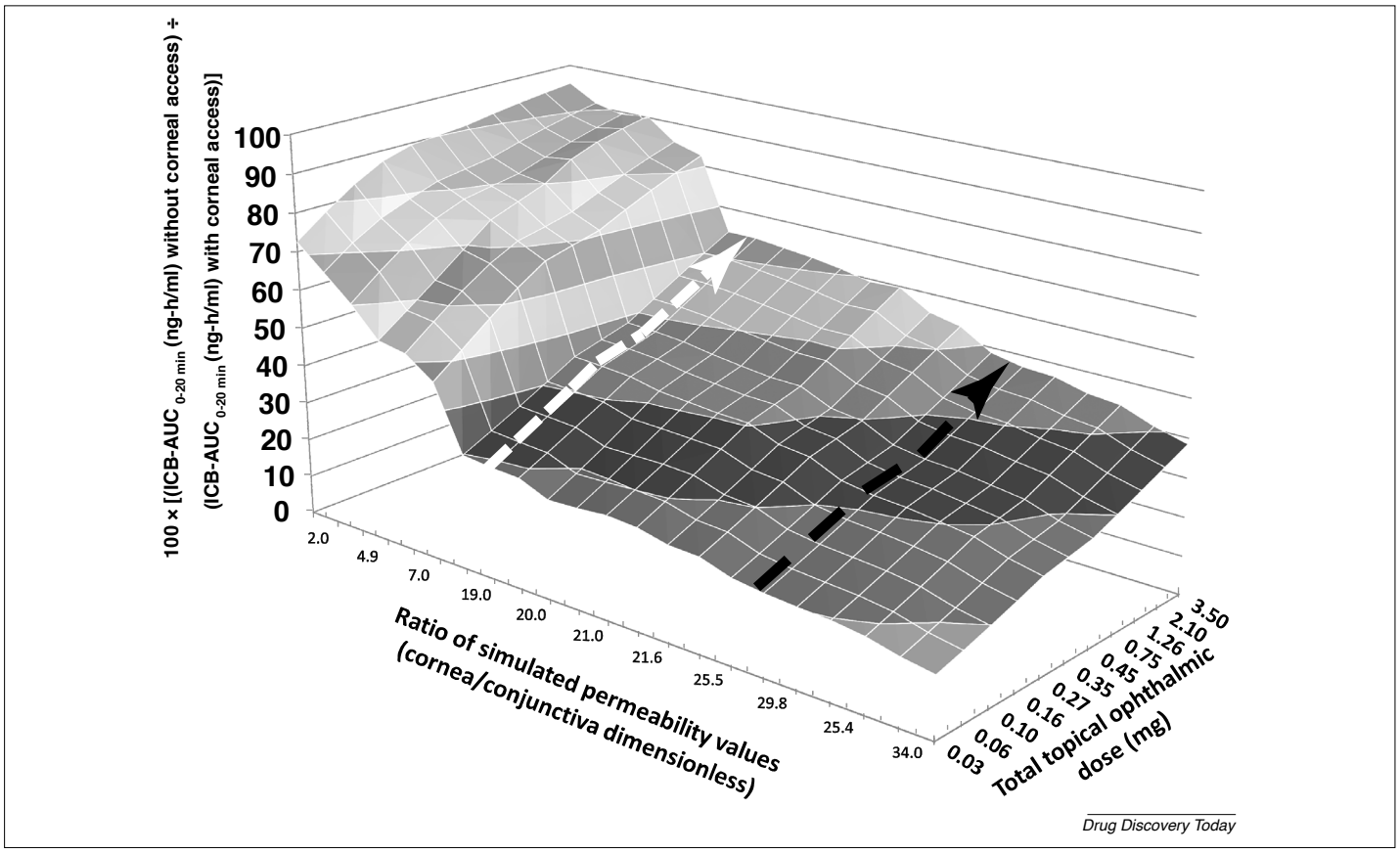


FIGURE 1

Topical ophthalmic biopharmaceutical model showing the relationship between simulated ocular epithelial tissue permeability (from 22 compounds in Table 1 in the main text), such as increasing ratio of simulated corneal to conjunctival permeability; simulated solubilized dose delivery (e.g., increasing fraction of dissolved dose available for absorption from precorneal space); as a function of predicted %total exposure in iris–ciliary body (ICB) attributed to blocking corneal access of instilled eye-drop [e.g., area under the curve (AUC) blocked/AUC access × 100 [7]). Formulation impact can be seen from the positive slope of the x-axis (dose, mg) versus the z-axis (ICB-AUC) for a simulated permeability ratio >10. For a simulated permeability ratio <10, the dose versus ICB-AUC appears saturable, which might be because of the limited ocular surface area available for absorption and precorneal residence time. The ratio in the z-axis increases putatively because of the influence of the noncorneal absorption of drugs, as a function of having a higher dissolved fraction of total dose available for absorption, as seen from novel topical formulations, such as those in CEQUA™ and INVELTYS™ (broken white and black arrows, respectively). The OCAT™ v3-'SplitFrontEye' human model takes into account all relevant systemic losses from the precorneal space, conjunctiva, and intraocular compartments. These compounds have been used to corroborate °BCS presented in Fig. 2 in the main text based on their known apparent corneal-permeability and conjunctival-permeability values and experimental ocular pharmacokinetics or pharmacodynamics reports [52,53,58,61,63,64,66,70,72,76,80,81,83,84]. Adapted from [52,53].

Ocular bioavailability from a topical eye drop is equal to the fraction of the dose absorbed (Fa) under conditions of minimal metabolism. Exceptions in Table 1 are topically applied prodrugs (i.e., latanoprost or hydrocortisone acetate), which undergo rapid and complete esterolysis during absorption, accounted for by estimating the Fa of their respective hydrolysis products. Proposed topical ophthalmic orthologs of dose number (Do) and permeation number (Pn) [54] were simulated as dose (mg, Fig. 1 x-axis) that would be dissolved in 0.03 ml eye drop volume, and the in-parallel (e.g., as opposed to in-series) conductance of the cornea and conjunctiva towards dissolved drug (dimensionless ratio obtained from OCAT™ PSA of predicted corneal and/or conjunctival permeability, Fig. 1, y-axis) [52,53]. Briefly, the Do of topical ophthalmic formulations would be the total dose:highest achievable pharmaceutical solubility ratio within a typical eye drop volume (Fig. 2, horizontal axis), and Pn would be a precorneal surface concentration profile over the in-parallel sum of absorption residence times through corneal and conjunctival epithelia (Fig. 2, vertical axis). Transconjunctival tissue conductance has a 6.6-fold difference between the lowest and highest apparent permeability examples (Table 1, ethoxzolamide versus apraclonidine), and an average ± standard error of the mean (S.E.M.) permeability of 5.03 ± 0.35 ($\times 10^{-6}$) cm/s for entire diverse data set. Values and range are in general agreement with a multivariate QSPR-based *in silico* model built for the prediction of conjunctival drug permeability [45]. Negligible differences could be attributed to the use of N-in-one [55] ocular cassette dosing in porcine tissues [45], versus single-drug substrate permeabilities estimated in freshly isolated rabbit conjunctivas (Table 1). By contrast, corneal tissue conductance displays a wider range of apparent permeability values [Table 1, from 0.4 to 96 ($\times 10^{-6}$) cm/s, ciprofloxacin to latanoprost, respectively, ~$2\log_{10}$ unit range] and higher degree of sensitivity towards molecular structural diversity. Hence, for each compound, a ratio of its OCAT™ PSA predicted corneal-to-conjunctival permeability (y-axis in Fig. 1 ranked in ascending order of corneal permeability) confirms that compounds with low corneal permeability are sensitive to the transconjunctival route of anterior segment absorption in the absence of corneal contact. A conductance ratio of ~10 (e.g., for every ten molecules that mass transfer

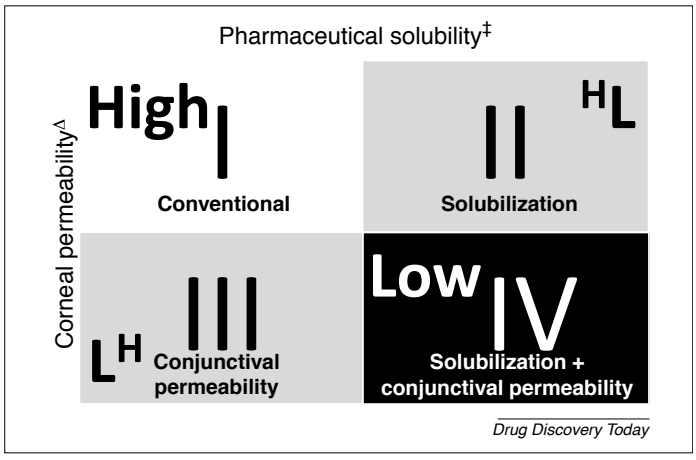


FIGURE 2

The ophthalmic biopharmaceutical classification system (°BCS) proposed here categorizes compounds into four classes. The horizontal axis (pharmaceutical solubility) refers to high and low formulation concentrations (^{H}L is high corneal permeability with low pharmaceutical solubility), related to the ratio of total dose within a single eye drop volume to highest achievable pharmaceutical solubility (e.g., ‡fraction of the dose, as [drug], available for absorption into either corneal or conjunctival tissue). The vertical axis refers to high and low apparent corneal permeability (L^{H} is low corneal permeability with high pharmaceutical solubility), where Δsuboptimal corneal permeability can be offset by sufficient conjunctival permeability in quadrants III and IV. Similarly, for quadrants II and IV, which describe compounds with low solubility, the °BCS assumes a formulation that is able to dissolve (denoted as solubilization) the putative drug in a way to make total dose completely available for absorption. Other assumptions include no influence from biochemical processes, such as efflux, active transport, or intra-epithelial tissue metabolism. Adapted from [52].

through the cornea, one is transferred through the conjunctiva) appears as an inflection point for changing the primary route of absorption into the eye from transcorneal to transconjunctival. Whereas compounds with a >10 cornea:conjunctival mass transfer ratio yield poor ICB exposures with a conjunctival access-only model configuration, at ratios <5, exposure in the ICB is attributed largely to transconjunctival flux. Furthermore, for compounds that prefer the transcorneal route, increasing the fraction of the dose in a topical solution formulation available for absorption shifts the preferred route of penetration of the drug towards the noncorneal (Fig. 1, observed positive slope in x-axis vs. z-axis).

Cyclosporine A (CsA) and loteprednol etabonate (LE), topical ophthalmic drugs with low (e.g., μg/ml) intrinsic solubility, formulated at 0.09% CEQUA™ [56] (OTX-101, 0.9 mg/ml CsA) and 1.0% INVELTYS™ [57] (KPI-121, 10 mg/ml LE) using novel solubilization technologies, showed increased conductance through the noncorneal route as a result of higher dose delivery with a greater fraction available for absorption by virtue of solubilization [52,53]. Broken arrows in Fig. 1 depict the simulated formulation effects on the mechanism of absorption contour for LE (an intrinsic corneal:conjunctival conductance ratio of 24, broken black line in Fig. 1) and CsA (an intrinsic corneal: conjunctival conductance ratio of 10, broken white line in Fig. 1), respectively, from a no- to high-formulation effect. These are anecdotal accounts illustrating the increased impact of transconjunctival flux on ICB exposure at higher solubilized doses. By this rationale, both INVELTYS™ and CEQUA™ can be classified as Class IV products delivering a relatively large (versus respective intrinsic solubilities) dose via the transconjunctival route of absorption, whereas LE and CsA both display higher inherent corneal tissue permeability [52,53].

Concluding remarks

Constraints associated with anatomical and physiological considerations for various routes of ocular drug delivery render strategies for ophthalmic research, development, and regulatory compliance complex and extensive. Although PK and pharmacodynamic (PD) considerations remain central for the targeted treatment of ocular disease, in a drug discovery environment that is becoming increasingly cost sensitive, the savings potential offered by aspiring, fully predictive, ocular biopharmaceutical computational models can be significant [12]. Objectives in building M&S software include comprehensive capture and retention of vetted empirical training data sets; however, validation of full predictive power (especially in the human eye) remains in its infancy [3,23]. Lagging emergence of non-invasive yet quantitative bioanalytical technologies for understanding pharmacologically active drug concentration within the globe of the eye evidenced after penetration from an eye drop, is just one of several limitations [46]. Ophthalmic M&S provides a means of improving quality in candidate selection from *de novo* discovery and accelerates the development of novel ocular dosage forms through innovative pragmatic assessment methods of efficiency or durability in drug delivery.

References

1 Ghate, D. and Edelhauser, H.F. (2006) Ocular drug delivery. *Expert Opin. Drug Deliv.* 3, 275–287
2 FDA (2018) FDA
3 Harigaya, Y. *et al.* (2018) Bioequivalence study methods with pharmacokinetic endpoints for topical ophthalmic corticosteroid suspensions and effects of subject demographics. *Pharm. Res.* 36, 13
4 Geroski, D.H. and Edelhauser, H.F. (2000) Drug delivery for posterior segment eye disease. *Invest. Ophthalmol. Vis. Sci.* 41, 961–964
5 Ghate, D. and Edelhauser, H.F. (2008) Barriers to glaucoma drug delivery. *J. Glaucoma* 17, 147–156
6 Lee, V.H. and Robinson, J.R. (1986) Topical ocular drug delivery: recent developments and future challenges. *J. Ocul. Pharmacol.* 2, 67–108
7 Ahmed, I. and Patton, T.F. (1985) Importance of the noncorneal absorption route in topical ophthalmic drug delivery. *Invest. Ophthalmol. Vis. Sci.* 26, 584–587
8 Ahmed, I. *et al.* (1987) Physicochemical determinants of drug diffusion across the conjunctiva, sclera, and cornea. *J. Pharm. Sci.* 76, 583–586
9 Wang, W. *et al.* (1991) Lipophilicity influence on conjunctival drug penetration in the pigmented rabbit: a comparison with corneal penetration. *Curr. Eye Res.* 10, 571–579
10 Conrad, J.M. *et al.* (1978) Influence of tonicity and pH on lacrimation and ocular drug bioavailability. *J. Parenter. Drug Assoc.* 32, 149–161
11 Patton, T.F. and Robinson, J.R. (1975) Influence of topical anesthesia on tear dynamics and ocular drug bioavailability in albino rabbits. *J. Pharm. Sci.* 64, 267–271
12 Shen, J. *et al.* (2018) Targeted ocular drug delivery with pharmacokinetic/pharmacodynamic considerations. *Pharm. Res.* 35, 217
13 Sieg, J.W. and Robinson, J.R. (1975) Vehicle effects on ocular drug bioavailability i: evaluation of fluorometholone. *J. Pharm. Sci.* 64, 931–936
14 Sieg, J.W. and Robinson, J.R. (1977) Vehicle effects on ocular drug bioavailability II: evaluation of pilocarpine. *J. Pharm. Sci.* 66, 1222–1228
15 Sieg, J.W. and Robinson, J.R. (1979) Vehicle effects on ocular drug bioavailability III: shear-facilitated pilocarpine release from ointments. *J. Pharm. Sci.* 68, 724–728
16 Maurice, D.M. (1953) The permeability of the cornea. *Ophthalmic Lit.* 7, 3–26

17 McCartney, H.J. *et al.* (1965) An autoradiographic study of the penetration of subconjunctivally injected hydrocortisone into the normal and inflamed rabbit eye. *Invest. Ophthalmol.* 4, 297–302
18 Wine, N.A. *et al.* (1964) The ocular uptake of subconjunctivally injected C14 hydrocortisone. 1. Time and major route of penetration in a normal eye. *Am J. Ophthalmol* 58, 362–366
19 Conrad, J.M. and Robinson, J.R. (1980) Mechanisms of anterior segment absorption of pilocarpine following subconjunctival injection in albino rabbits. *J. Pharm. Sci.* 69, 875–884
20 Doane, M.G. *et al.* (1978) Penetration routes of topically applied eye medications. *Am. J. Ophthalmol.* 85, 383–386
21 Lee, V.H. and Robinson, J.R. (1979) Mechanistic and quantitative evaluation of precorneal pilocarpine disposition in albino rabbits. *J. Pharm. Sci.* 68, 673–684
22 Miller, S.C. *et al.* (1981) A physiologically based pharmacokinetic model for the intraocular distribution of pilocarpine in rabbits. *J. Pharmacokinet. Biopharm.* 9, 653–677
23 Rodrigues, G.A. *et al.* (2018) Topical drug delivery to the posterior segment of the eye: addressing the challenge of preclinical to clinical translation. *Pharm. Res.* 35, 245
24 Bouzom, F. *et al.* (2012) Physiologically based pharmacokinetic (PBPK) modelling tools: how to fit with our needs? *Biopharm. Drug Dispos.* 33, 55–71
25 Jiang, W. *et al.* (2011) The role of predictive biopharmaceutical modeling and simulation in drug development and regulatory evaluation. *Int. J. Pharm.* 418, 151–160
26 Kostewicz, E.S. *et al.* (2014) PBPK models for the prediction of in vivo performance of oral dosage forms. *Eur. J. Pharm. Sci.* 57, 300–321
27 Shah, V.P. *et al.* (2015) A science based approach to topical drug classification system (TCS). *Int. J. Pharm.* 491, 21–25
28 Bloom, C. *et al.* (2010) Presented at Controlled Release Society (CRS) Annual Meeting, Poster #366. *Mucoadhesive nanoparticles and global modeling for topical ocular drug delivery*
29 Kovacs, K. *et al.* (2012) Pharmacokinetic study of vitreous and serum concentrations of triamcinolone acetonide after posterior sub-Tenon's injection. *Am. J. Ophthalmol.* 153, 939–948
30 Friedrich, S. *et al.* (1997) Drug distribution in the vitreous humor of the human eye: the effects of intravitreal injection position and volume. *Curr. Eye Res.* 16, 663–669
31 Friedrich, S. *et al.* (1997) Finite element modeling of drug distribution in the vitreous humor of the rabbit eye. *Ann. Biomed. Eng.* 25, 303–314
32 Friedrich, S. *et al.* (1997) Drug distribution in the vitreous humor of the human eye: the effects of aphakia and changes in retinal permeability and vitreous diffusivity. *J. Ocul. Pharmacol. Ther.* 13, 445–459
33 Missel, P.J. *et al.* (2010) Simulating dissolution of intravitreal triamcinolone acetonide suspensions in an anatomically accurate rabbit eye model. *Pharm. Res.* 27, 1530–1546
34 Prausnitz, M.R. and Noonan, J.S. (1998) Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. *J. Pharm. Sci.* 87, 1479–1488
35 Edward, A. and Prausnitz, M.R. (2001) Predicted permeability of the cornea to topical drugs. *Pharm. Res.* 18, 1497–1508
36 Edwards, A. and Prausnitz, M.R. (1998) Fiber matrix model of sclera and corneal stroma for drug delivery to the eye. *AIChE J.* 44, 214–225
37 Kidron, H. *et al.* (2010) Prediction of the corneal permeability of drug-like compounds. *Pharm. Res.* 27, 1398–1407
38 Fu, X.C. and Liang, W.Q. (2002) A simple model for the prediction of corneal permeability. *Int. J. Pharm.* 232, 193–197
39 Clark, D.E. (1999) Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 2. Prediction of blood-brain barrier penetration. *J. Pharm. Sci.* 88, 815–821
40 Clark, D.E. (1999) Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. *J. Pharm. Sci.* 88, 807–814
41 Huang, H.-S. *et al.* (1983) Corneal penetration behavior of β-blocking agents III: in vitro–in vivo correlations. *J. Pharm. Sci.* 72, 1279–1281
42 Huang, H.-S. *et al.* (1983) Corneal penetration behavior of β-blocking agents II: assessment of barrier contributions. *J. Pharm. Sci.* 72, 1272–1279
43 Schoenwald, R.D. and Huang, H.-S. (1983) Corneal penetration behavior of β-blocking agents I: physicochemical factors. *J. Pharm. Sci.* 72, 1266–1272
44 Ramsay, E. *et al.* (2018) Corneal and conjunctival drug permeability: systematic comparison and pharmacokinetic impact in the eye. *Eur J. Pharm. Sci.* 119, 83–89
45 Ramsay, E. *et al.* (2017) Impact of chemical structure on conjunctival drug permeability: adopting porcine conjunctiva and cassette dosing for construction of in silico model. *J. Pharm. Sci.* 106, 2463–2471
46 Maurice, D.M. (2002) Drug delivery to the posterior segment from drops. *Surv. Ophthalmol.* 47 (Suppl. 1), S41–S52
47 Li, S.K. and Hao, J. (2018) Transscleral passive and iontophoretic transport: theory and analysis. *Expert Opin. Drug Deliv.* 15, 283–299
48 Geroski, D.H. and Edelhauser, H.F. (2001) Transscleral drug delivery for posterior segment disease. *Adv. Drug Deliv. Rev.* 52, 37–48
49 Pitkanen, L. *et al.* (2005) Permeability of retinal pigment epithelium: effects of permeant molecular weight and lipophilicity. *Invest. Ophthalmol. Vis. Sci.* 46, 641–646
50 Delamere, N.A. (2005) Ciliary body and ciliary epithelium. *Adv. Organ Biol.* 10, 127–148
51 Shirasaki, Y. (2008) Molecular design for enhancement of ocular penetration. *J. Pharm. Sci.* 97, 2462–2496
52 Gukasyan, H.J. (2018) Presented at 255th ACS National Meeting, MEDI 268. *Optimizing ophthalmic drug delivery strategies to enhance efficacy: modeling, formulation technology, and °BCS*
53 Gukasyan, H.J. (2016) Presented at the Ophthalmic Drug Development Summit www.hansonwade.com, Washington DC, July 26–28. *An extension of the BCS and druggability rules of thumb to the eye*
54 Sugano, K. and Terada, K. (2015) Rate- and extent-limiting factors of oral drug absorption: theory and applications. *J. Pharm. Sci.* 104, 2777–2788
55 Laitinen, L. *et al.* (2003) N-in-one permeability studies of heterogeneous sets of compounds across Caco-2 cell monolayers. *Pharm. Res.* 20, 187–197
56 FDA (2018) *CEQUA™ (Cyclosporine Ophthalmic Solution) 0.09%, for Topical Ophthalmic Use*. FDA
57 FDA (2018) *INVELTYS™ (Loteprednol Etabonate Ophthalmic Suspension) 1%, for Topical Ophthalmic Use*. FDA
58 Huang, H.S. *et al.* (1983) Corneal penetration behavior of beta-blocking agents III: In vitro-in vivo correlations. *J. Pharm. Sci.* 72, 1279–1281
59 Schoenwald, R.D. and Huang, H.S. (1983) Corneal penetration behavior of beta-blocking agents I: physiochemical factors. *J. Pharm. Sci.* 72, 1266–1272
60 Shoghi, E. *et al.* (2013) Solubility-pH profiles of some acidic, basic and amphoteric drugs. *Eur J. Pharm. Sci.* 48, 291–300
61 Palkama, A. *et al.* (1985) Comparison of the effects of adrenergic agonists and alpha-, beta 1-, beta 2-antagonists on the intraocular pressure and adenylate cyclase activity in the ciliary processes of the rabbit. *Acta Ophthalmol.* 63, 9–15
62 Scozzafava, A. *et al.* (1999) Carbonic anhydrase inhibitors. Synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J. Med. Chem.* 42, 2641–2650
63 Loftsson, T. *et al.* (1994) Topically effective ocular hypotensive acetazolamide and ethoxyzolamide formulations in rabbits. *J. Pharm. Pharmacol.* 46, 503–504
64 Chien, D.S. *et al.* (1990) Corneal and conjunctival/scleral penetration of p-aminoclonidine, AGN 190342, and clonidine in rabbit eyes. *Curr. Eye Res.* 9, 1051–1059
65 Human Metabolome Database (HMDB); http://www.hmdb.ca/ [Accessed 26th March 2019].
66 Costagliola, C. *et al.* (2003) Ocular perfusion pressure and visual field indice modifications induced by alpha-agonist compound (clonidine 0.125%, apraclonidine 1.0% and brimonidine 0.2%) topical administration. An acute study on primary open-angle glaucoma patients. *Ophthalmologica* 217, 39–44
67 McFarland, J.W. *et al.* (2001) Estimating the water solubilities of crystalline compounds from their chemical structures alone. *J. Chem. Inf. Comput. Sci.* 41, 1355–1359
68 DrugBank. https://www.drugbank.ca/ [Accessed 26th March 2019].
69 Bhagav, P. *et al.* (2010) Development and validation of stability indicating UV spectrophotometric method for the estimation of brimonidine tartrate in pure form, formulations and preformulation studies. *Pharm. Lett.* 2, 106–122
70 Zhang, Y. *et al.* (2013) Development of inclusion complex of brinzolamide with hydroxypropyl-beta-cyclodextrin. *Carbohydr. Polym.* 98, 638–643
71 ChemIDplus Advanced. https://chem.nlm.nih.gov/chemidplus/ [Accessed 26th March 2019].
72 Tai, M.C. *et al.* (2003) Corneal and scleral permeability of quinolonesl—a pharmacokinetics study. *J. Ocul. Pharmacol. Ther.* 19, 547–554
73 Breda, S.A. *et al.* (2009) Solubility behavior and biopharmaceutical classification of novel high-solubility ciprofloxacin and norfloxacin pharmaceutical derivatives. *Int. J. Pharm.* 371, 106–113
74 Dannenfelser, R. and Yalkowsky, S.H. (1989) Database for aqueous solubility of nonelectrolytes. *Comput. Appl. Biosci.* 5, 235–236
75 Apt, L. *et al.* (1979) Patient compliance with use of topical ophthalmic corticosteroid suspensions. *Am. J. Ophthalmol.* 87, 210–214
76 Leibowitz, H.M. *et al.* (1978) Evaluation of dexamethasone acetate as a topical ophthalmic formulation. *Am. J. Ophthalmol.* 86, 418–423

77 Maren, T.H. *et al.* (1990) Chemical and pharmacological properties of MK-927, a sulfonamide carbonic anhydrase inhibitor that lowers intraocular pressure by the topical route. *Exp. Eye Res.* 50, 27–36
78 Diehl, H. and Markuszewski, R. (1985) Studies on fluorescein-II The solubility and acid dissociation constants of fluorescein in water solution. *Talanta* 32, 159–165
79 ALOGPS 2.1. http://www.vcclab.org [Accessed 26th March 2019].
80 Pawar, P. *et al.* (2013) Topical ocular delivery of fluoroquinolones. *Expert Opin. Drug Deliv.* 10, 691–711
81 Vale, J. *et al.* (1972) Topical propranolol and ocular tension in the human. *Br. J. Ophthalmol.* 56, 770–775
82 He, Y. *et al.* (2003) Solubilization of fluasterone in cosolvent/cyclodextrin combinations. *Int. J. Pharm.* 264, 25–34
83 Worda, C. *et al.* (2001) Treatment of keratoconjunctivitis sicca with topical androgen. *Maturitas* 37, 209–212
84 Ahmed, I. *et al.* (1989) The kinetics of timolol in the rabbit lens: implications for ocular drug delivery. *Pharm. Res.* 6, 772–778