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Research Article

Development of Novel *In Silico* Model to Predict Corneal Permeability for Congeneric Drugs: A QSPR Approach

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This study was undertaken to determine *in vivo* permeability coefficients for fluoroquinolones and to assess its correlation with the permeability derived using reported models in the literature. Further, the aim was to develop novel QSPR model to predict corneal permeability for fluoroquinolones and test its suitability on other training sets. The *in vivo* permeability coefficient was determined using cassette dosing (*N-in-One*) approach for nine fluoroquinolones (norfloxacin, ciprofloxacin, lomefloxacin, ofloxacin, levofloxacin, sparfloxacin, pefloxacin, gatifloxacin, and moxifloxacin) in rabbits. The correlation between corneal permeability derived using *in vivo* studies with that derived from reported models was determined. Novel QSPR-based model was developed using *in vivo* corneal permeability along with other molecular descriptors. The suitability of developed model was tested on β-blockers ($n = 15$). The model showed better prediction of corneal permeability for fluoroquinolones ($r^2 > 0.9$) as well as β-blockers ($r^2 > 0.6$). The newly developed QSPR model based upon *in vivo* generated data was found suitable to predict corneal permeability for fluoroquinolones as well as other sets of compounds.

1. Introduction

Topical route is the oldest and convenient mode of drug administration for ophthalmic disorders. However, several precorneal factors such as lacrimal secretion (tear turn over and reflux tearing), tear protein binding, pH, shorter contact time, and other corneal constraints limit drug penetration [1]. Moreover, presence of various uptake and efflux transporters in cornea also reposted to further complicate the ocular bioavailability [2]. Therefore, developing drug specifically for ophthalmic use is of paramount importance.

Conventionally, the drugs intended for oral administrations have been developed in consideration of their physicochemical properties. This enables to enhance the pharmacokinetic properties, efficacy and reduces the toxicity of lead compound. In drug discovery exploitation of *in silico* techniques to expedite the process of lead optimization in drug discovery is rampant. Among such techniques, Quantitative Structure Property Relationship (QSPR) approach correlates the biological activity of a molecule with its physicochemical properties through variety of descriptors. In recent years, QSPR approach has been exploited for the development of models to predict penetration of drugs across physiological barriers such as CNS, blood, and intestinal [3].

The drugs used for ophthalmic indications are arbitrarily developed from the oral or systemic indications, rather than systematically ocular specific studies. To date, very few

drugs have been designed, developed, and studied for ocular specific use. However, the ocular pharmacokinetics of a drug is known to be different and complicated compared to any other indications [4]. Thus, in this context a drug intended for oral use is expected to behave differently when used empirically for the topical application. As physicochemical properties such as lipophilicity, molecular size, charge, degree of ionization, solubility, and pH, affect the rate and extent of corneal permeability [5].

In ophthalmology, fluoroquinolones are most widely used antimicrobial agents. These agents were initially discovered, designed, and developed for systemic infections and further extended for ophthalmic use. Their broad-spectrum activity, bactericidal property, better ocular penetration, and relative safety embark its use in ophthalmology. Most of the fluoroquinolones for topical use lack regress understanding of their corneal penetration, residence time, required spectrum, optimum frequency of usage, and corneal toxicity despite of their widespread use in ophthalmics [6]. Therefore, an emphasis on QSPR strategy to optimize ocular pharmacokinetic properties along with antimicrobial efficacy is desirable [7].

Previously reported QSPR models to predict corneal permeability for congeneric and noncongeneric drugs have been developed based upon *in vitro* studies. A weak correlation is reported to exist between the *in vitro* and *in vivo* studies [8, 9]. Eventually their applicability of these models to predict *in vivo* corneal penetration remains unclear.

In present study we developed novel QSPR based *in silico* model to predict corneal permeability for fluoroquinolones based upon *in vivo* generated permeability coefficient along with molecular descriptors. The applicability of previously reported models based upon *in vitro* data to predict corneal permeability for fluoroquinolone was also studied. Furthermore, the generated QSPR model was evaluated for its aptness using the other training sets (β-blockers).

2. Materials and Methods

2.1. Chemicals. Pure samples of norfloxacin, ciprofloxacin HCl, gatifloxacin, and lomefloxacin HCl were generously gifted by Dr. Reddy Labs (Hyderabad, India), Lupin Labs (Pune, India), Sun Pharmaceuticals (Mumbai, India), and Organics Ltd., (Hyderabad, India), respectively. Gratis samples of ofloxacin, pefloxacin mesylate, and sparfloxacin were obtained from Cipla Ltd., (Mumbai, India). Moxifloxacin and levofloxacin were obtained as a generous gift from Capital Pharma (Baddi, India). Other chemicals used in the study were of analytical grade and procured from standard drug companies.

2.2. Preparation of the Cocktail Formulations. A total of nine fluoroquinolones (Table 1) were randomly divided into 2 groups wherein, group A consists of ofloxacin, sparfloxacin, pefloxacin mesylate, and gatifloxacin and group B consists of norfloxacin, ciprofloxacin HCl, lomefloxacin HCl, levofloxacin, and moxifloxacin. In both groups, individual fluoroquinolones were dissolved at concentration of 0.1%. Thus, the total fluoroquinolones concentration in group A and group B were 0.4% and 0.5%, respectively. Sterile boric acid (1.9% w/v) in water was used as the aqueous media and the final formulations were filtered using 0.22 μ filter and autoclaved further before use.

2.3. In Vivo Transcorneal Permeation of Fluoroquinolones Using Cassette Dosing (N-in-One). Study protocol and experimental procedures were approved by the Institutional Animal Ethics Committee of All India Institute of Medical Sciences (AIIMS), New Delhi, India. New Zealand albino rabbits of either sex (1.5–2.0 kg) were obtained from Central Animal Facility, AIIMS. Animals were housed at standard laboratory conditions temperature-controlled room at 24 ± 2°C and humidity 55 ± 15% and given food and water *ad libitum*. All experiments were performed in accordance to the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research.

The sterile cocktail formulations of group A and group B were individually instilled (50 μL) on the lower fornix of rabbit eyes ($n = 4$) with the help of a calibrated micropipette. Aqueous paracentesis was performed under the influence of topical anesthesia at 5, 15, 30, 60, 120, and 240 min after the instillation of cocktail formulation. For each time point, a volume amounting to 50 μL of aqueous humor was aspirated through the corneal surface. The aqueous humor samples obtained were stored at −80°C until quantification by HPLC.

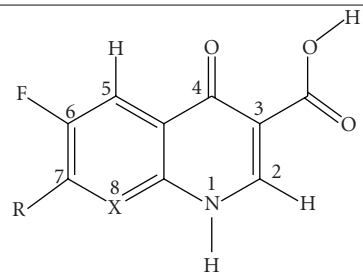
2.4. Quantification of Fluoroquinolones in Aqueous Humor Using HPLC. A Thermo Finnigan (Thermo Electron Corporation, USA) HPLC equipped with degasser, quaternary pump, autosampler, and PDA detector was employed for quantification of fluoroquinolones in samples. In the chromatographic quantification, analytical separation was performed using a C18 Symmetry Shield column (4.6 × 150 mm, 5 μm, Waters, USA) under a gradient flow of methanol, acetonitrile, and potassium phosphate buffer (20 mM, pH 2.5) at different ratio and time. For each spiked compound an external calibration curve was plotted and the analyte's spectra was assessed by matching them with custom made PDA spectra of inbuilt library of Chromquest version 4 (Thermo Electron Corporation, USA).

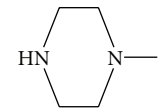
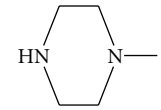
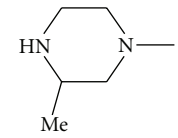
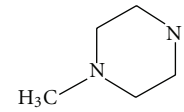
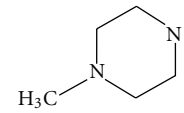
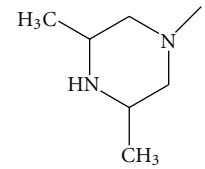
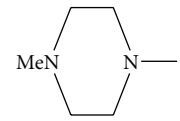
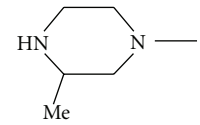
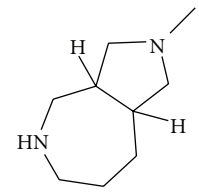
Samples were deproteinized with pure acetonitrile (ratio of 1 : 2 v/v), vortexed, and centrifuged at 3500 g for 10 min. The supernatant was vacuum concentrated at 40°C for a stipulated time. The dried concentrate was reconstituted with 100 μL mixture of water and acetonitrile (1 : 1), and 20 μL of the obtained supernatant was injected for quantification.

2.5. Applicability of Reported Models to Predict In Vivo Corneal Permeability for Fluoroquinolones. Available literature reveals existence of two models correlating the corneal permeability of compounds with their physiochemical properties. Therefore, present study evaluated the suitability of these reported models to predict *in vivo* corneal penetration for fluoroquinolones.

2.5.1. Evaluation of Model 1 Reported by Yoshida and Topliss. The first model reported by Yoshida and Topliss [10]

Table 1: Structures of various fluoroquinolones used in the study.



Compound	X	R_1	R_5	R_7
Norfloxacin	C	C_2H_5	H	
Ciprofloxacin	C	c-C_3H_5	H	
Lomefloxacin	C-F	C_2H_5	H	
Ofloxacin	Fused C-1-8 (O) Cyclic ring		H	
Levofloxacin	Fused C-1-8 (O) Cyclic ring		H	
Sparfloxacin	C-F	c-C_3H_5	NH_2	
Pefloxacin	C	C_2H_5	H	
Gatifloxacin	–C–OCH_3	c-C_3H_5	H	
Moxifloxacin	–C–OCH_3	c-C_3H_5	H	

was based upon two molecular descriptors, $\Delta \log P$ and $\log D$ to predict corneal permeability coefficient (logPC). Algorithm (1) is proposed to predict corneal permeability for noncongeneric compounds

$$\text{logPC} = -0.404(\pm\ 0.114)\Delta \log P + 0.141(\pm\ 0.090)\log D - 3.862(\pm\ 0.451), \quad (1)$$

wherein logPC denotes permeability coefficient, $\Delta \log P$ expresses the difference between the octanol-water partition coefficients ($\log P_{\text{octanol}}$) and alkane-water partition coefficients ($\log P_{\text{alkane}}$), and $\log D$ denotes dissociation constant

As reported, $\Delta \log P$ was calculated using LOGPSTAR software in training sets of 32 diverse noncongeneric compounds including steroids and β-blockers. Since LOGPSTAR was currently unavailable and obsolete. Therefore, experimentally derived $\Delta \log P$ was used for all fluoroquinolones. $\Delta \log P$ was derived as the difference in $\log P$(o/w) to $\log P$(cyclohexane/water) by using shake flask method given in OECD guidelines of Chemical Testing (No. 107 and 117). The other molecular descriptor $\log D$ was calculated using

$$\log D(\text{pH}) = \log P - \log\left(1 + 10^{*(\text{pka}-\text{pH})}\right). \quad (2)$$

$\log D$ denotes dissociation constant and $\log P$ denotes partition coefficient.

Both, molecular descriptors ($\Delta \log P$ and $\log D$) were derived for the studied fluoroquinolones (norfloxacin, ciprofloxacin, lomefloxacin, ofloxacin, levofloxacin, sparfloxacin, pefloxacin, gatifloxacin, and moxifloxacin) and logPC was determined using Algorithm (1). The derived logPC from Algorithm (1) was correlated with that obtained from controlled *in vivo* experiment in rabbits.

2.5.2. Evaluation of Model 2 Reported by Fu and Liang. Model 2 evaluated reported by Fu and Liang [11] was based upon charge and molecular volume as the molecular descriptors to predict logPC. Algorithm (3) was reported to predict corneal permeability for noncongeneric compounds

$$\text{logPC} = -5.566Q_H^2 + 3.027Q_H - 0.155Q_{O,N} - 9.413 \times 10^{-4}\,V - 4.278 \quad (3)$$

wherein logPC denotes permeability coefficient, Q_H is sum of the absolute values of net atomic charge of hydrogen atom, and $Q_{O,N}$ is sum of the absolute values of the net atomic charges of oxygen and nitrogen atoms. V is molecular volume.

The charge (Q_H^2,Q_H,$Q_{O,N}$) for all fluoroquinolones was calculated by using GAMESS software and molecular volume by drug design software developed at Super Computing Facility, Indian Institute of Technology, New Delhi. Structures of all fluoroquinolones were drawn HYPERCHEM version 11.0 and optimized the geometry to lowest energy state. The net charge on each atom was calculated as the sum of all atoms present in the particular structure. The molecular descriptors used in algorithm (3) were derived for the studied nine fluoroquinolones to predict logPC. The derived logPC from algorithm (3) was also correlated with *in vivo* experimentally obtained logPC.

2.6. Generation of the Novel QSPR Model to Predict In Vivo Corneal Permeability. A novel QSPR model was generated using pooled molecular descriptors determined using *in vitro, in vivo,* and *in silico* approaches for fluoroquinolones.

In Vitro Data. Partition coefficient ($\log P$) was determined using OECD Shake Flask method for chemical testing (No. 107 and 117) using biphasic system of octanol/water at 25°C and 37°C.

In Vivo Data. Permeability coefficients (logPC) were obtained from controlled *in vivo* experiment conducted in rabbits using cassette dosing (*N-in-One*) approach.

In Silico Data. Different molecular descriptors were generated using softwares like CAChe Scientific (Fujitsu version 6.1.12.33, Japan), ACD/Chemsketch (Freeware version 10), ChemDraw and Chem BioDraw Ultra from Cambridge Soft (trial version), calculator Plugins of Chem Axon's Marvin version 5.2.5.1, Chem Axon Ltd. All fluoroquinolone structures were drawn in respective workspace and standard molecular mechanics were run before geometry optimization. The descriptors like molecular weight, $\log P$, topological polar surface area (TPSA), molar refractivity, number of "H"-bond donors/acceptor, dipole moment, lowest unoccupied molecular orbitals (LUMO), highest unoccupied molecular orbital (HUMO), GAP, molecular volume, connolly accessible area, connolly molecular area, principal moment, dipole moment, molecular weight, wiener index, melting point, polar surface area, number of rotatable bonds and molar refractivity, molar volume, polarizibility, parachor, index of refraction, surface tension, density, monoisotopic mass, nominal mass, average mass, apKa, and charge on the each atom, that is, Q_N, Q_O, N, Q_H, Q_F and Q_C were extracted using different kind of softwares.

2.7. Suitability of Newly Developed QSPR Model to Predict Corneal Permeability of Compounds Other Than Fluoroquinolones. To ensure the suitability of newly developed QSPR model, the congeneric compounds used by Yoshida and Topliss [10] and Fu and Liang [11] were pooled. All the congeneric compounds (β-blockers, $n = 15$) were pooled and molecular descriptors used in newly developed QSPR model were extracted. The β-blockers pooled include acebutolol, alprenolol, atenolol, betaxolol, bevantolol, bufuralol, levobunolol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propanolol, sotalol, and timolol. The logPC derived using newly developed QSPR model was correlated with reported logPC for all the β-blockers.

3. Results

3.1. In Vivo Transcorneal Permeation Using Cassette Dosing Approach. Two different HPLC-PDA method were developed and validated to elute all fluoroquinolones in group A

Table 2: Gradient mobile phase used during the analysis by HPLC-PDA for fluoroquinolones in group A and group B.

Time (min)	Methanol (%)	Acetonitrile (%)	Buffer (20 mM) (%)	Flow rate (mL/min)
		Group A		
0.01	0	10	90	1.0
4.00	5	15	85	1.0
10.0	0	40	60	1.0
12.0	0	10	90	1.0
		Group B		
0.01	10	10	80	1.0
6.00	10	10	80	1.0
8.00	0	50	50	1.0
10.0	10	10	80	1.0

and B. For the analysis of group A (ofloxacin, sparfloxacin, pefloxacin, and gatifloxacin) the gradient mobile phase consisted of different ratios of methanol, acetonitrile, and potassium phosphate buffer (20 mM, pH 2.5) over the period of 12 min (Table 2). For the analysis of fluoroquinolones in group B (norfloxacin, ciprofloxacin, lomefloxacin, levofloxacin, and gatifloxacin) the gradient mobile phase consisted of different ratios of methanol, acetonitrile, and potassium phosphate buffer (20 mM, pH 2.5) over the period of 10 min (Table 2).

The representative chromatograms of all fluoroquinolones eluted in group A and group B are shown in Figures 1(a) and 1(b).

The validation parameters regarding the accuracy and precision were found to be within the allowable limits according to ICH guidelines for HPLC in bioanalysis. The mean concentration versus time plot for all fluoroquinolones (group A and B) are shown in Figures 2(a) and 2(b).

The derived pharmacokinetics parameters like Cmax, Tmax, AUC, logPC at 30 min and 240 min generated for all fluoroquinolones are tabulated in Table 3.

3.2. Applicability of Existing Models to Predict Corneal Permeability for Fluoroquinolones

3.2.1. Evaluation of Model 1 Reported by Yoshida and Topliss. Both molecular descriptors ($\Delta \log P$ and $\Delta \log D$) derived for fluoroquinolones in algorithm (1) to derive logPC showed a weak Spearman correlation (0.133) with that obtained from *in vivo* experiment (Table 4).

The weak correlation was observed at 30 min ($r^2 = 0.0699$) and 240 min ($r^2 = 0.0137$). The statistically insignificant correlation suggests that algorithm (1) is unable to appropriately predict corneal permeability for fluoroquinolones (Figure 3(a)).

3.2.2. Evaluation of Model 2 Reported by Fu and Liang. Algorithm (3) reported by Fu and Liang [11] was employed to derive logPC for all studied fluoroquinolones (Table 4). A very weak Spearman correlation of 0.134 was observed between the logPC derived experimentally at both 30 min and 240 min with that logPC derived using algorithm (3) (Figure 3(b)). The statistically insignificant correlation suggests that algorithm (3) is unable to appropriately predict corneal permeability for fluoroquinolones.

3.3. Generation of New QSPR Model for Determination of In Vivo Corneal Permeability. A total of 72 molecular descriptors were extracted for all nine topically studied fluoroquinolones (norfloxacin, ciprofloxacin, lomefloxacin, ofloxacin, levofloxacin, sparfloxacin, pefloxacin, gatifloxacin and moxifloxacin) using *in vitro, in vivo,* and *in silico* approaches. All sets of data were subjected to multilinear regression (MLR) statistical analysis by Sigma Stat Software (version 3.5, Germany). For the generation of algorithm the *in vivo* data of logPC calculated for absorption phase (30 min) and elimination phase (240 min) was used. The algorithms developed with $\log P$ and apKa either with GAP (algorithms (4) and (5)) and or with TPSA (algorithms (6) and (7)) exhibited good correlation

$$\begin{aligned}\text{logPC 30 min} &= 13.972 + (1.529 * \log P) \\ &\quad - (1.375 * \text{apKa}) + (1.308 * \text{GAP}), \\ \text{R} = 0.908 \quad &\text{Rsqr} = 0.825 \quad \text{Adj Rsqr} = 0.720,\end{aligned} \tag{4}$$

$$\begin{aligned}\text{logPC 240 min} &= 9.946 + (1.368 * \log P) \\ &\quad - (1.429 * \text{apKa}) + (0.952 * \text{GAP}) \\ \text{R} = 0.940 \quad &\text{Rsqr} = 0.883 \quad \text{Adj Rsqr} = 0.813,\end{aligned} \tag{5}$$

wherein logPC denotes logarithm of permeability coefficient, $\log P$ is partition coefficient at water 25 ± 1°C for 30 min, GAP is the difference in HUMO and LUMO energy levels, and apKa is acid dissociation constant.

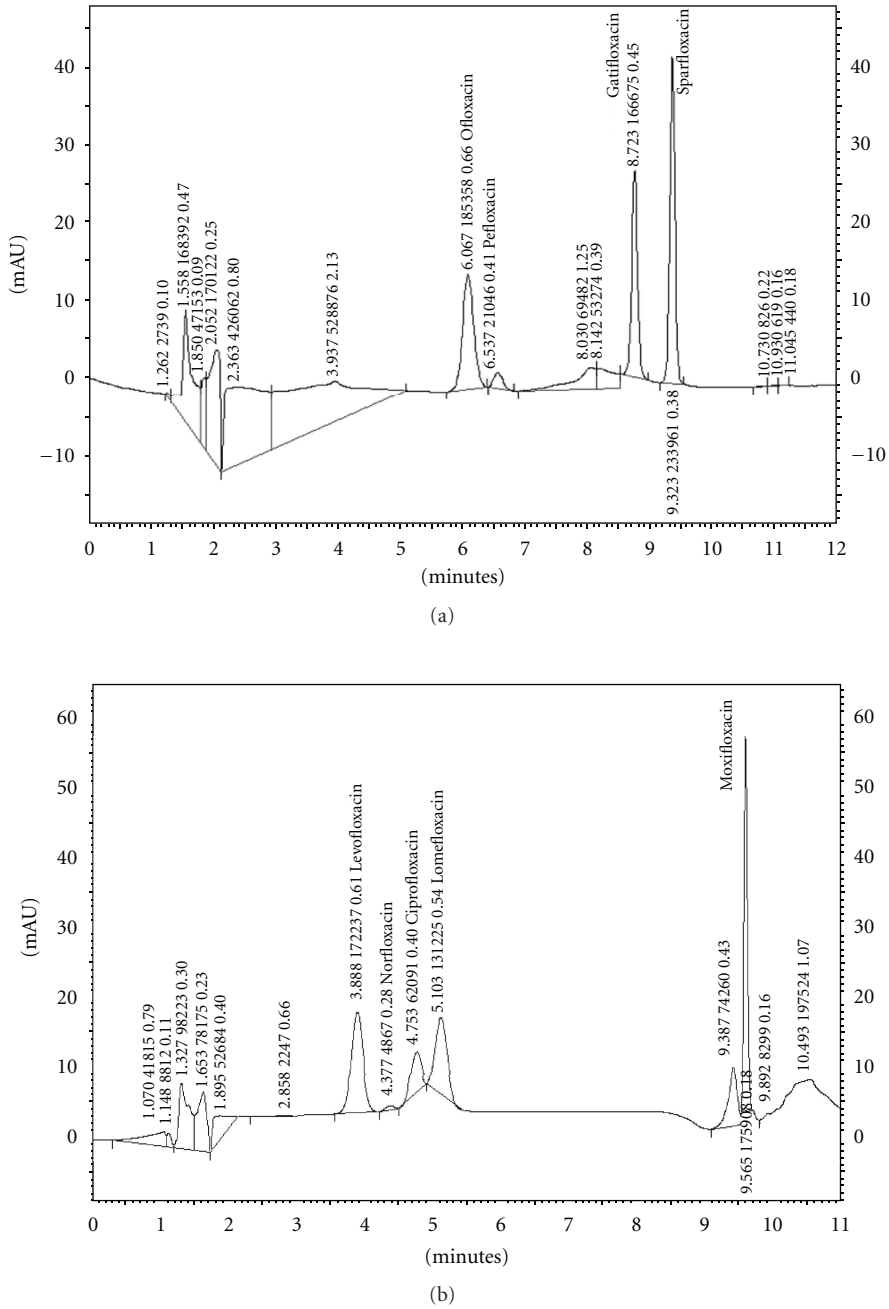


Figure 1: (a) Chromatogram of the fluoroquinolones in group A (ofloxacin, pefloxacin, gatifloxacin, and sparfloxacin) eluted in single run. (b) Chromatogram of fluoroquinolones in group B (levofloxacin, norfloxacin, ciprofloxacin, lomefloxacin, and moxifloxacin) eluted in single run.

Algorithms (6) and (7) were developed using logP, TPSA, and apKa as molecular descriptor at 30 min and 240 min.

$$\log PC\ 30\ \text{min} = -1.453 + (1.726 * \log P) - (0.708 * \text{apKa}) + (0.0104 * \text{TPSA})$$

$$\text{R} = 0.934 \quad \text{Rsqr} = 0.872 \quad \text{Adj Rsqr} = 0.796, \tag{6}$$

$$\log PC\ 240\ \text{min} = -2.726 + (1.439 * \log P) - (0.672 * \text{apKa}) + (0.00421 * \text{TPSA})$$

$$\text{R} = 0.937 \quad \text{Rsqr} = 0.877 \quad \text{Adj Rsqr} = 0.803, \tag{7}$$

wherein logPC denotes logarithm of permeability coefficient, $\log P$ as partition coefficient at water 36 ± 1°C for 5 min,

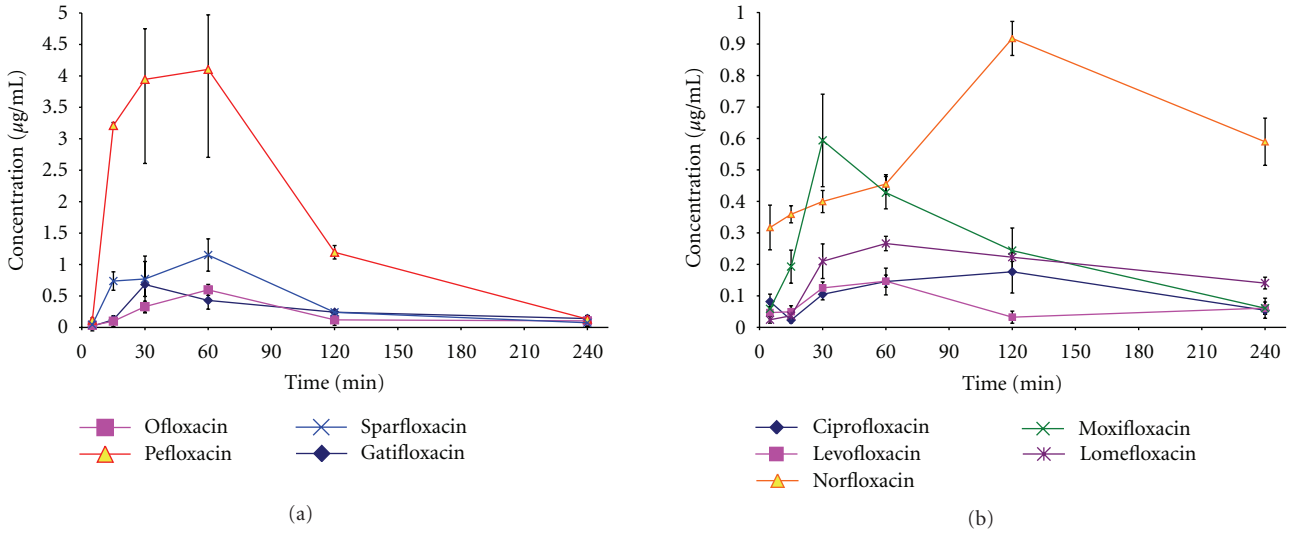


Figure 2: (a) Mean concentration (μg/mL) versus time (min) plot for all the fluoroquinolones in group A (ofloxacin, pefloxacin, gatifloxacin, and sparfloxacin). (b) Mean concentration (μg/mL) versus time (min) plot for all the fluoroquinolones in group B (levofloxacin, norfloxacin, ciprofloxacin, lomefloxacin, and moxifloxacin).

Table 3: Pharmacokinetic parameters derived for all fluoroquinolones using *in vivo* cassette dosing approach in rabbits.

Fluoroquinolones	Cmax ± SD (μg/mL)	Tmax (min)	AUC (μg·hr/mL)	logPC (30 min)	logPC (240 min)
Norfloxacin	0.456 ± 0.059	60 min	2.854	−5.051	−6.382
Ciprofloxacin HCl	0.146 ± 0.084	60 min	0.507	−5.806	−7.089
Lomefloxacin HCl	0.266 ± 0.046	60 min	0.833	−5.519	−6.859
Ofloxacin	0.597 ± 0.736	60 min	0.930	−5.284	−6.723
Levofloxacin	0.147 ± 0.053	60 min	0.311	−5.678	−7.225
Sparfloxacin	1.151 ± 0.514	30 min	1.770	−4.753	−6.396
Pefloxacin mesylate	4.103 ± 1.399	60 min	7.225	−4.075	−5.778
Gatifloxacin	0.682 ± 0.904	30 min	1.179	−5.006	−6.628
Moxifloxacin	0.594 ± 0.294	30 min	1.045	−5.022	−6.664

TPSA as topological polar surface area, apKa is acid dissociation constant.

Figure 4 shows the pictorial representation of newly developed QSPR model using fluoroquinolone as model group.

In the four newly developed algorithms (algorithms (4), (5), (6), and (7)) log P and apKa are common molecular descriptors, signifying the key descriptors affecting penetration of fluoroquinolones across the cornea. Algorithms (4), (5), (6), and (7) showed a positive correlation with log P and inverse correlation with apKa. TPSA and GAP also showed a positive correlation with permeability coefficient in all newly developed algorithms.

3.4. Suitability of New QSPR Model to Predict Corneal Permeability for Compounds Other Than Fluoroquinolones. The logPC was generated for β-blockers pooled from Models 1 and 2. Newly developed QSPR model showed a positive Spearman correlation of 0.831 and 0.887 between the newly developed algorithms (4) and (5) with already reported logPC (Figure 5(a)).

A positive Spearman correlation of 0.881 and 0.803 between the logPC generated for β-blockers using newly developed algorithms (6) and (7) and reported logPC (Figure 5(b)).

4. Discussion

Conventionally, antimicrobial drugs developed and approved for systemic infections are extended for ocular infections. An antimicrobial agent having good corneal penetration and efficacy is desired in preventing sight threatening infections. Fluoroquinolones are the commonly used topical antimicrobial agents in ocular therapeutics. It is well known that less than 5% of the topically applied drug penetrates through the cornea. Therefore, there is an urgent need to understand the constraints exerted by the eye for the development of an ocular specific antimicrobial

Table 4: Determination of various molecular descriptors used in Model 1, and 2 along with permeability coefficient (logPC) derived using algorithms (1) and (3) for all fluoroquinolones.

Fluoroquinolones	$\Delta\log P$ (Cyclohexane-Octanol)	log$D$	logPC$^{\$}$	Q_H	Q_H^2	$Q_{O,N}$	Molecular Volume	logPC$^{\#}$
Norfloxacin	0.051	−0.493	−3.752	0.876	0.767	−2.954	110.375	−5.540
Ciprofloxacin HCl	−0.358	−0.963	−3.653	0.848	0.719	−2.750	108.000	−5.388
Lomefloxacin HCl	1.113	0.182	−4.086	0.847	0.717	−2.577	113.635	−5.412
Ofloxacin	0.759	−0.290	−4.009	0.461	0.212	−2.567	121.375	−3.781
Levofloxacin	0.734	−0.186	−3.985	0.461	0.212	−2.567	121.375	−3.781
Sparfloxacin	1.025	0.174	−4.052	1.648	2.715	−3.704	125.875	−13.946
Pefloxacin mesylate	0.709	−0.095	−3.962	0.472	0.223	−2.321	113.375	−3.836
Gatifloxacin	0.526	−0.399	−3.931	0.815	0.663	−2.697	123.125	−5.203
Moxifloxacin	0.908	−0.272	−4.067	0.821	0.674	−2.774	130.750	−5.239

$^{\$}$logPC derived from algorithm (1) from Model 1; $^{\#}$logPC derived from algorithm (3) from Model 2.

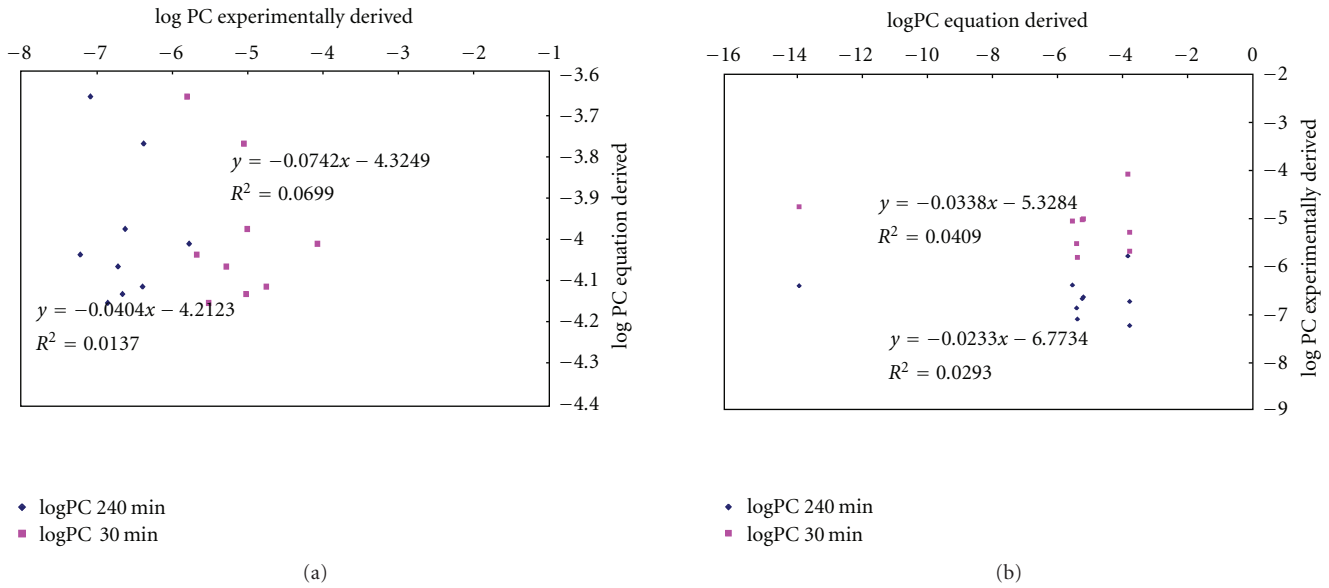


Figure 3: (a) Permeability coefficient (logPC) derived using algorithm (1) versus permeability coefficient (logPC) derived from *in vivo* study. (b) Graph between permeability coefficient (logPC) derived using algorithm (3) versus permeability coefficient (logPC) derived from *in vivo* study.

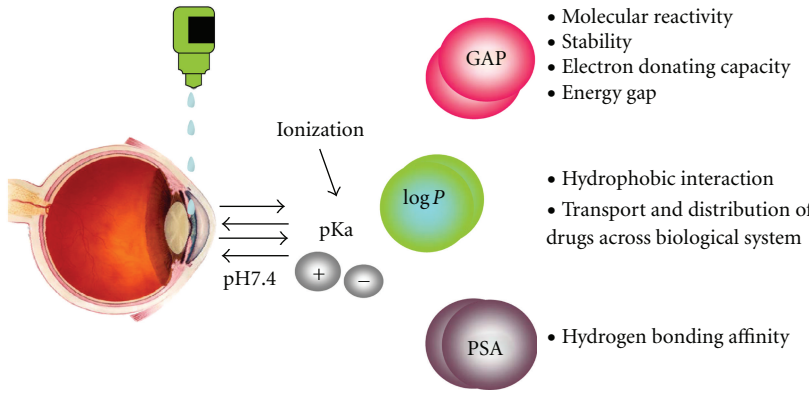


Figure 4: Pictorial representation of the newly developed QSPR model depicting various factors affecting the penetration of topically applied drugs.

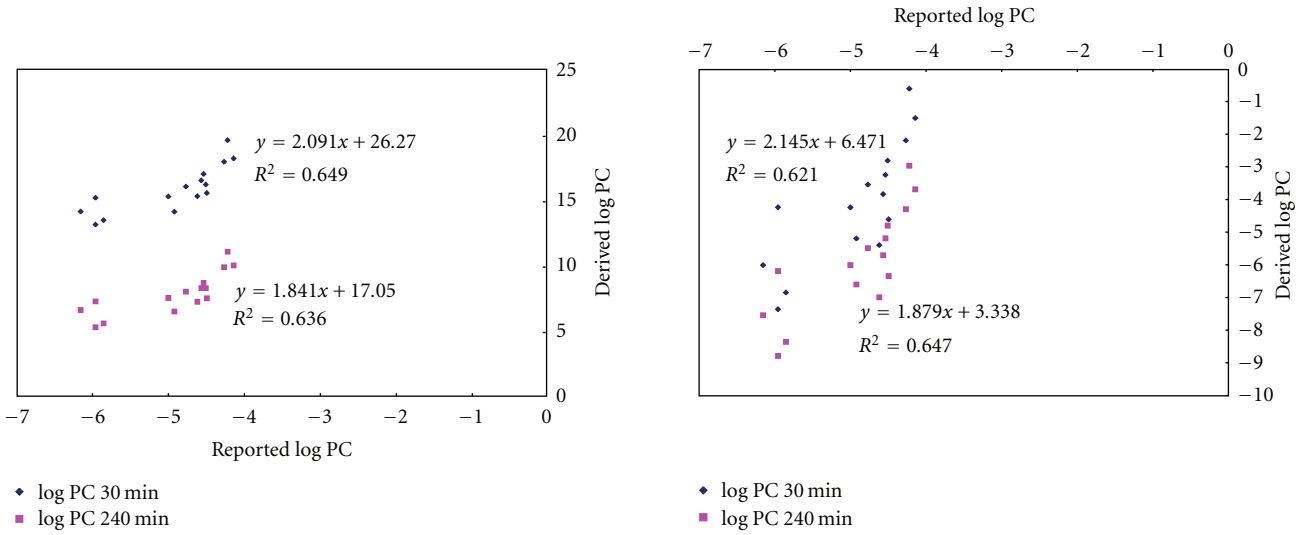


Figure 5: (a) Correlation between permeability coefficient (logPC) reported in literature for β-blockers versus permeability coefficient (logPC) derived values using newly developed algorithms (4) and (5) for β-blockers ($n = 15$). (b) Correlation between the permeability coefficient (logPC) reported in the literature for β-blockers versus permeability coefficient (logPC) derived values from newly developed algorithms (6) and (7) for β-blockers ($n = 15$).

agent [3]. In present study, QSPR approach has been used to comprehend the physicochemical factors affecting corneal permeability for topically applied drugs. An attempt was made in present study to evaluate the suitability of *in silico* models in predicting the *in vivo* corneal permeability of fluoroquinolones. To ascertain the obtained results, various molecular descriptors were extracted from different softwares. Additionally, *in vivo* corneal permeability was determined in rabbits employing cassette dosing approach, a high throughput pharmacokinetic screening technique which has been exploited for various indication [12–14]. So far this approach has been limited to *in vitro* and few *in vivo* models in ophthalmic drug research [15–17]. However, for the first time, we employed cassette dosing technique to study the *in vivo* pharmacokinetic profile of topically applied fluoroquinolones and to derive a controlled *in vivo* permeability coefficient

Nine topically used fluoroquinolones were dissolved in two cassettes: group A with four and group B with five fluoroquinolones. Being weakly basic in nature fluoroquinolones are expected to exist in ionized form at pH 4.5. Therefore, pH of both formulations was maintained at 5.0 and osmolarity as 307 mOsM/L using boric acid. Boric acid (1.9%) has been reported to be an appropriate vehicle for the preparation of ophthalmic solutions having basic nature [18]. In addition, it is reported to avoid any interaction between fluoroquinolones and di/monovalent cations [19].

In the literature two models have been reported to predict the corneal permeability. The first model was reported by Yoshida and Topliss [10] based upon $\Delta \log P$ and $\log D$ as molecular descriptors. In this model, $\Delta \log P$ mainly correlates with the solute hydrogen bonding acidity or basicity and to lesser extend dipolarity or polarizability. The second model was reported by Fu and Liang [11] was based upon charge and molecular volume as the molecular descriptors. Unfortunately, both the earlier reported models were unsuitable to predict *in vivo* corneal permeability of fluoroquinolones. A poor correlation between the permeability coefficient derived using reported models [10, 11] with that derived from present *in vivo* study was observed. This may be due to the fact that reported models were developed based upon permeability coefficients (logPC) pooled from various *in vitro* studies. The *in vitro* conditions denote a static system rather than actual dynamic conditions which exists in eye. Moreover, the involvement of various drug transporters and precorneal factors is not considered in such studies and are also believed to have major role. Thus, reported models [10, 11] based upon *in vitro* corneal permeability are unable to correlate with *in vivo* corneal permeability for fluoroquinolones. Therefore, a new *in silico* model based upon *in vivo* permeability coefficient data was felt desirable. Also the *in silico* model should be able to correlate with parameters like ionizing property, transporter susceptibility, and lipophilicity, precorneal pH of compound.

A novel QSPR model was developed based upon *in vivo* corneal permeability along with molecular descriptors like $\log P$, apKa, GAP, and TPSA. All the molecular descriptors used are known to affect the corneal permeability. Civiale and coworkers [20] also reported that corneal permeation process can be confined at the ocular epithelium layer. Studies have been reported that in corneal epithelium the drugs are expected to undergo transcellular pathway

[21, 22]. Whereas the penetrating molecules are expected in hydrophilic form as a result of pH or after hydrolysis due to enzymes (by prodrug attempt) to cross hydrophilic stroma. Thus, the hydrogen bonding affinity plays a vital role in transcorneal permeation.

The dissociation constant (pKa) of the compound determines the HLB (hydrophilic/lipophilic balance) in the cornea. Thus, pKa provides a correlation with the corneal permeability of topically applied drug. Based upon the presence of ionizable/functional groups molecules can posses more than one pKa. An assumption was made in the newly developed QSPR model that for fluoroquinolones apKa has more implication on the availability of the species. Other studies also report that corneal penetration can be enhanced by selecting the drug molecule with appropriate pKa and offering optimal lipid solubility [23, 24]. In general, adjusting pH so that a drug is mostly in the unionized form increases its lipophilicity and thus, its transcellular permeability, and ocular absorption.

Another molecular descriptor GAP, which is difference in energy between E_{HUMO} and E_{LUMO}, has also reported to be an important stability index and related to transporters susceptibility [25]. The E_{HUMO} measures the electron donating and E_{LUMO} measures electron accepting property of the molecule. A HUMO and LUMO energy separation has been used as a conventional measure of kinetic stability for various π-electron systems. A large GAP has been reported to relate with high stability for a molecule in the sense of its lower sensitivity in chemical reaction [26, 27].

TPSA is defined as the sum of surfaces of polar atoms in a molecule and known to correlate with transporter susceptibility. TPSA makes use of functional group based upon large database of structures that avoids the need to calculate ligand 3D structure or to decide which one is relevant biological conformation. Therefore, in this analysis, TPSA has been taken into account to define the transcorneal penetration of congeneric compounds. Fernandes and coworkers [28] suggested that compounds with high TPSA are transported, while those with low TPSA are not. Moreover, conjugation to compounds like GSH is reported to increase the TPSA values as a favoring transport mechanism. A strong correlation between TPSA and transport properties has been observed in present study. The algorithm developed either with TPSA or GAP proved to predict the intraocular penetration appropriately as compared with other molecular descriptors tried with highest degree of correlation ($r^2 > 0.9$)

The applicability of the newly developed algorithms (4), (5), (6), and (7) on other sets of compounds apart from fluoroquinolones was also tested. The congeneric compound trial sets taken for this analysis belong to the category of β-blockers where the ioniziability and pH-dependent changes in the lipophilicity are not much of concern. Their structures predominately lacking primary amino groups or carboxylic acids (except atenolol having primary amine) therefore, pH-induced changes are not expected to play a major role. This study further evaluated the applicability of the developed algorithm on the noncongeneric compounds using similar training sets. However, the corneal predictability was found to be insignificant. The newly developed *in silico* model is based upon *in vivo* data and hence may predict corneal permeability for congeneric compounds other than fluoroquinolone more appropriately.

5. Conclusions

The previously reported algorithms based on *in vitro* data failed to predict *in vivo* corneal permeability for fluoroquinolones. A novel QSPR model consisting of four new algorithms were developed using GAP, TPSA, log*P,* and apKa as molecular descriptors to predict *in vivo* corneal permeability. The hypothesis generated showed high degree of applicability to predict transcorneal penetration as it was based upon the *in vivo* corneal permeability coefficients data. Moreover, the developed model was also found to predict corneal permeability of the congeneric β-blockers ($r^2 > 0.6$) reported in the literature. Further studies are in progress to evaluate its utility in large number of other congeneric compounds.

Abbreviations

apKa:	Acid dissociation constant
EDTA:	Ethylene diamine tetra acetic acid
EHUMO:	Energy for highest unoccupied molecular orbitals
ELUMO:	Energy for lowest unoccupied molecular orbitals
GAMESS:	General atomic and molecular electronic structure system
GAP:	Difference in $E_{\mathrm{HUMO}} - E_{\mathrm{LUMO}}$
HPLC:	High-performance liquid chromatography
log *P*:	Logarithm of partition coefficient
logPC:	Logarithm of permeability coefficient
log *D*:	Logarithm of dissociation constant
OECD:	Organization for economic cooperation and development
QSPR:	Quantitative structure property relationship
TPSA:	Topological polar surface area.

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