Diffusion and Monod kinetics model to determine In vivo Human Corneal Oxygen-Consumption Rate During Soft Contact Lens Wear.

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Funding

We thank to Ministerio de Educación y Ciencia (MEC) of Spain for financial support through project ENE2011-24761. LFdC is grateful to SEP-CONACYT for financial support through grant 154626 and project UNAM-DGAPA IN-102512. SIH is grateful to Red Temática de la Materia Condensada Blanda-CONACYT for a postdoctoral fellowship.

Conflicts of interest

The authors have no conflicts of interest to declare.

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ABSTRACT

Purpose: We present an analysis of the corneal oxygen consumption Q_c from non linear models, using data of oxygen partial pressure or tension (p_{O2}) obtained from *in vivo* estimation provided by Bonanno et al.[1]

Methods: Assuming that the cornea is a single homogeneous layer, the oxygen permeability through the cornea will be the same regardless of the type of lens that is available on it. The obtention of the real value of the maximum oxygen consumption rate $Q_{c,max}$, is very important because this parameter is directly related with the gradient pressure profile into the cornea and moreover, the real corneal oxygen consumption is influenced by both anterior and posterior oxygen fluxes.

Results: Our calculations give different values for the maximum oxygen consumption rate $Q_{c,max}$, when different oxygen pressure values (high and low p_{O2}) are considered at the interface cornea-tears film.

Conclusion: Present results are relevant for the calculation on the partial pressure of oxygen, available at different depths into the corneal tissue behind contact lenses of different oxygen transmissibility.

Keywords: Corneal oxygen consumption; corneal oxygen permeability; monod kinetics model; corneal oxygen pressure.

INTRODUCTION

The rate of oxygen consumption in the cornea is an important parameter to guarantee its physiology, and it may be influenced by the use of contact lenses over the cornea. Estimation of tear oxygen pressure or tension (p_c) behind hydrogel lenses in humans, using a time-domain phosphorescence measurement system, allowed to obtain the oxygen consumption from established oxygen diffusion models.[1] Hovewer, previous papers have calculated oxygen consumption kinetics from transient post-lens tear-film oxygen tension, a method that relies on the simplistic assumption of a constant corneal-consumption rate that leads to negative oxygen tensions in the cornea which lacks physical meaning.[2,3] Since oxygen diffusivity and consumption in the human cornea have not been directly measured, some authors, such as Larrea et al.,[4] Alvord et al.,[5] and Chhabra et al.,[2] have proposed mathematical models of time-dependent oxygen diffusion that allows the estimation of corneal consumption and diffusivity. Such authors make use of the nonlinear Monod kinetics model to describe the local oxygen-consumption rate. Nevertheless, although the consumption of oxygen is a result of corneal cell metabolism that depends on a great number of factors, Chhabra et al. assume that the oxygen consumption only depends on the partial pressure of oxygen, and use as parameters $Q_{c,max}$, $(Dk)_c$ and K_m. Here $Q_{c,max}$ is the maximum corneal oxygen-consumption rate, k_c is the corneal oxygen solubility, and D_c is the corneal oxygen diffusion coefficient. K_m is the methabolic or Monod dissociation equilibrium constant, and is a parameter in the Monod kinetic model, which determines the shape of the Q_c vs. p_c curve, and represents the oxygen pressure when the aerobic metabolism in the cornea reaches the 90 % of the maximum oxygen consumption.[6,7]

The appropriate relationship between oxygen consumption and p_c into the cornea should be continuous, yielding a value of zero consumption when p_c is zero. Moreover, oxygen consumption should increase with increasing p_c until the saturation level is reached. Considering this, we proceeded with the analysis of the oxygen consumption using non linear models, and also using data from *in vivo* estimations of partial oxygen pressure at the interface cornea-lens, provided by Bonanno (*personal communication*).

This work aims to present a single mathematical one-dimensional model of time-dependent oxygen diffusion through the cornea. The experimental data provided by Bonanno et al.[1] were used to validate the methabolic model used previously by Chhabra et al.,[2] and then to determine the oxygen consumption and methabolic constant K_m. For this purpose, similar to Chhabra et al.,[2] we fitted the model to three different cases of contact lenses: Balafilcon A, Polymacon1 (60 microns) and Polymacon2 (200 microns). In our calculations the oxygen permeability through the corneal tissue is considered constant, independent of the lens material situated onto the cornea, and the maximum oxygen consumption rate is also independent of the soft contact lens weared.

With the present work we intend to evaluate the impact of consider other values of $Q_{c,max}$, taking into account that K_m=2.2 mmHg, which is the value reported by Chhabra et al.[2] in the Monod kinetics model. From the values obtained for the parameter $Q_{c,max}$, the oxygen pressure profile into the cornea has been calculated for the open eye and closed eye conditions. Finally, we have established a comparison with the oxygen tension profiles given by Chhabra et al.[2]

METHODS

The non-steady state diffusion equation that gives oxygen tension as a function of time and position, for homogeneous slab of oxygen-consuming tissue (assuming a one-dimensional model for the cornea), is given by:

$$\frac{\partial^2 p_c}{\partial x^2} - \left(\frac{Q}{Dk}\right)_c = \frac{1}{D}\frac{\partial p_c}{\partial t} \qquad (1)$$

where, $p_c(x)$ is the partial pressure or tension of oxygen into the cornea, D_c is the diffusion coefficient of oxygen in the tissue (cm^2/sec), k_c is the oxygen solubility coefficient in the cornea tissue, i.e. Henry's law constant (cm^3 of O_2 /cm^3 layer/mm of Hg), and x is the distance perpendicular to the surface (cm). Q_c is the oxygen consumption rate in the cornea (mL of O_2/cm^3 of tissue layer /sec), and t is time (sec). In steady-state conditions, Eq. (1) becomes

$$\frac{\partial^2 p_c}{\partial x^2} = \left(\frac{Q}{Dk}\right)_c \qquad 0 \le x \le x_c \qquad (2)$$

As we have mentioned, the aerobic metabolism is quantified by the Monod kinetics model, also known as Michaelis Menton model,[5,8] which relates the oxygen consumption with the oxygen tension by mean of the expression,

$$Q_c(p_c) = \frac{Q_{c,\max} \cdot p_c(x)}{K_m + p_c(x)} \qquad (3)$$

where K_m is the Monod dissociation equilibrium constant to which we have referred above. For low oxygen partial pressure ($p_c \ll K_m$), the oxygen consumption rate depends linearly on the oxygen tension, and tends to zero when the oxygen pressure approaches to zero. For large oxygen pressure ($p_c \gg K_m$), the consumption also will be dependent on the oxygen tension, and it tends to a maximum value when the pressure is equal to 155 mm Hg at sea level, in the case of open eyes. In such situation $Q_c(p_c$=155 mmHg)= $Q_{c,max}$.

By mean of the nonlinear Monod kinetics model, Chhabra et al.,[2] obtain different values for the maximum corneal oxygen-consumption rate $Q_{c,max}$, depending on the contact lenses weared (see Table 1). The average of the values obtained is $Q_{c,max(ave)}$ =1.05x10^{-4} mL·cm^{-3}·s^{-1}. This value is 2.34 times higher than the one given by Brennan,[7] which is $Q_{c,max}$ =4.48x10^{-5} mL·cm^{-3}·s^{-1}. Furthermore, Chhabra et al.[2] propose a value of 2.2 mmHg for the K_m constant in Eq.(3) indicating that, in this case,

$\frac{Q_c}{Q_{c,\max}} = 0.9$ when p_c=20 mmHg, as we have already mentioned. This value of K_m has been given taken into account that the oxygen partial pressure for reaching 90% of the saturation oxygen consumption rate for various organism, is in the range of 12-25 mmHg.

Table 1. Parameters optimized by Chhabra et al.[2]

Lens	**$Q_{c,max.}$ 10^{-4} (mL(STp)$\cdot$cm$^{-3}\cdot$s^{-1})**	**K_m (mmHg)**	**$D_c\cdot 10^{-5}$ (cm^2/s)**	**$k_c\cdot 10^{-5}$ (mL/cm^3/mmHg)**	**$(Dk)_c$ (Barrer)[a]**
Balafilcon[b]	1.2	2.2	6.2	2.3	140
Polymacon1[c]	0.9	2.2	5.9	1.5	90

(a) 1 Barrer = 10^{-11} (cm^2/s)(mL O_2 (STp)/cm^3 /mmHg); (b) 100 μm of thickness. (c) 60 μm of thickness. All this parameters has been optimized (see Table III in Reference 2).

From our opinion such value of K_m is only valid for the assummed pressure value of 20 mmHg in Chhabra´s work. If we take the extreme values of 12 or 25 mmHg for the oxygen partial pressure (Shoup,[9] Amberson[10], Fatt,[11] Tang,[12] Takahashi et al.[13]), other values for K_m could have been possible. Nevertheless, the Chhabra´s value for K_m tends to be an estimated average value, and in this way it may be perfectly acceptable. For this reason we have used the value of K_m=2.2 mmHg obtained by Chhabra et al.,[2] in order to be able to stablish a comparison.

Anyway, our greater rejection to the results given by Chhabra et al.[2], is the use of two values for the corneal oxygen permeability, 140 and 90 barrers, when onto the cornea is weared a Balafilcon or a Polymacon lens, respectively (see Table 1). This is clear evidence that the values obtained by Chhabra et al.[2] when fitting the experimental data provided by Bonanno et al.[1], should be reviewed. These values are in disagreement with the value of the cornea oxygen permeability used by the researchers during the last 30 years, which are of 24.5 barres or 28.5 barrers.[5]

Taking into account that the 78% of the cornea is composed basically for water, we have considered the apparent oxygen permeability through the cornea tissue as the value of the oxygen permeability in water at temperature of 35ºC. This can be estimated as the product of the oxygen diffusion (D_{O_2}(water)=3.0 x10^{-5} cm^2/sec) and the oxygen solubility in water (k=3.1x10^{-5} $cm^3$of O_2/cm^3 mmHg).[14] Thus, as a result, we have used the value of 93 Barrers for the cornea oxygen permeability $(Dk)_c$. For this reason, the values of the parameters in Monod kinetic model should be revisited and new fits should be obtained.

Bonnano et al.[1] have determined the partial pressure of oxygen p_c at the interface cornea-lens, using phosphorescence dye technique in the observation of the variation of oxygen partial pressure as a function of time, from steady state between close eye condition to open eyes condition. The analysis of the experimental transitory, in combination whith the Eq.(1), have permitted to Bonanno et al.,[1] and Chhabra et al.[2] obtain the value of $Q_c(p_c)$ in different situations. In this paper, following a similar procedure to that of Chhabra et al.[2], we have obtained *in vivo* human corneal oxygen-consumption rate from the data reported by Bonanno et al.[2], in their measurements of oxygen tension at the postlens-tear film as a function of time. In the APPENDIX we show the technical procedure followed for solving the partial differential equation (PDE), using FiPy (http://www.ctcms.nist.gov/fipy) wich is a finite volume PDE solver written in Python[15].

RESULTS

The noninvasive *in vivo* experimental data provided by Bonanno et al.[1] allowed to determine oxygen consumption rate and diffusivity of the human cornea.[2,4] Considering the Monod kinetics model, and based on the experimental data given by Bonnano et al.,[1] our calculations of the maximum corneal oxygen-consumption rate ($Q_{c,max}$), are reported in Table 2, for the systems: cornea+Balafilcon lens, cornea+Polymacon1 lens and cornea+Polymacon2 lens.

Table2. Values of $Q_{c,max}$ obtained fitting the curves showed in Figures 1, 2 and 3 using the Equations 1 and 3.

Lens	**$Q_{c,max.}$ 10^{-4} (mL(STp)·cm^{-3}·s^{-1})**	**K_m (mmHg)**	**D_c·10^{-5} (cm^2/s)**	**K_c·10^{-5} (mL/cm^3/mmHg)**	**$(Dk)_c$ (Barrer)**
Balafilcon[a]	1.4	2.2	3.0	3.1	93
Polymacon1[b]	0.9	2.2	3.0	3.1	93
Polymacon2[c]	0.7	2.2	3.0	3.1	93

(a) 100 μm of thickness, (b) 60 μm of thickness, (c) 200 μm thickness. In this work we have only optimized de $Q_{c,max}$ parameter. The rest or parameters have been taken from literature, as we point out through the text.

In Figure 1-left we plot the postlens tear-film oxygen tension as a function of time for Balafilcon lens at 35ºC, using the reactive diffusion model described and used in the work by Chhabra et al.[2] In our calculations the cornea is assummed as a single homogeneous layer where the oxygen consumption rate represents an average of the oxygen consumption of three main layers (endothelium, stroma and epithelium). The lens is considered as a separated phase without oxygen consumption surrounded by two thin films of tears (prelens and postlens-tear films), where the resistence to the oxygen flux can be consider negligible because of their thickness (5 to 15 microns), in comparisson with the lens thickness (60 and 200 microns).[16]

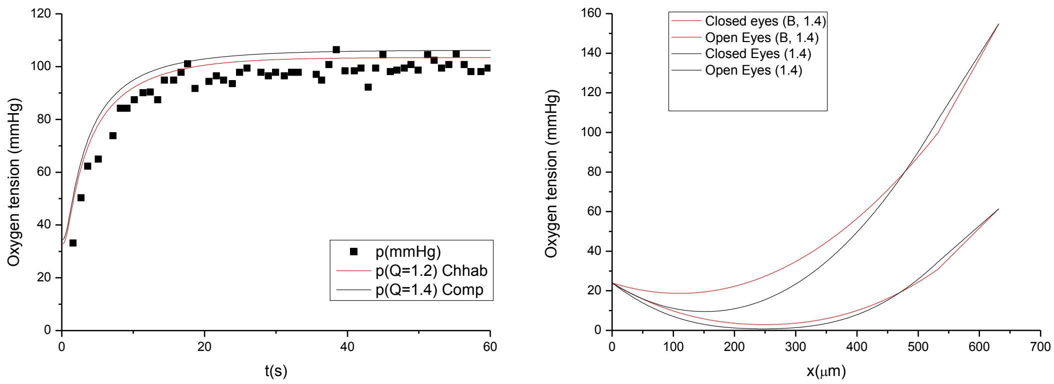

Figure 1. (Left) Representative results for the tear-film oxygen tension after 5 minutes of close eyes (CE) lens wear and **(right):** the steady state oxygen tension profile through cornea thickness. Red color is the fit to Bonanno data proposed by Chhabra et al.[2] Black color corresponds to our best fit to same data. Symbols are from Bonanno et al.[1], when the system is composed by cornea+Balafilcon lens.

A closer inspection of Figure 1-left shows that the values of the parameters found by Chhabra et al. (Table 1) to fit the experimental data of postlens tear-film oxygen tension as a function of time at the interface corneal lens (red line), on wearing Balafilcon lens from Bonanno et al.[1] data, appears as a best fit compared to the one achieved by us (black line), with parameters in Table 2 for both, open and closed eyes conditions. This is clearly related to the value of oxygen corneal oxygen permeability considered by Chhabra et al.[2] (140 Barrer considered by them, instead of the 93 Barrer considered by us). However, in the case of cornea-Polymacon1 lens and cornea-Polymacon2 lens systems, the behavior of our calculated curve is arguably similar than Chhabra´s curve, as can be seen in Figures 2 and 3.

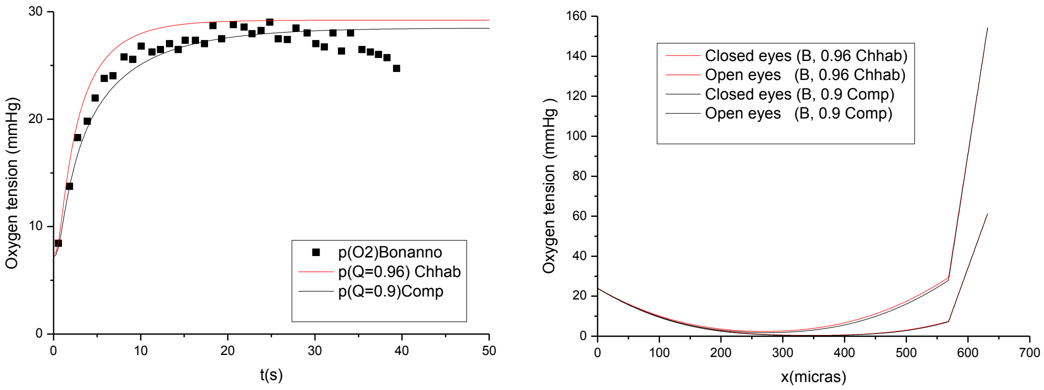

Figure 2. (Left) Representative results for the tear-film oxygen tension after 5 minutes of CE lens wear and **(right):** the steady state oxygen tension profile through cornea thickness. Red color is the fit to Bonanno data proposed by Chhabra et al.[2] Black color corresponds to our best fit to same data. Symbols are from Bonanno et al.[1], when the system is composed by cornea+Polymacon1 lens.

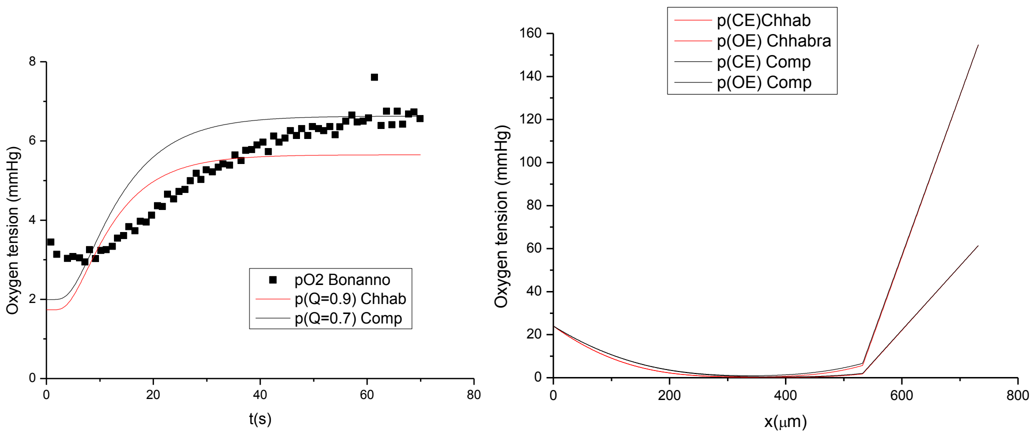

Figure 3. (Left) Representative results for the tear-film oxygen tension after 5 minutes of CE lens wear and **(right):** the steady state oxygen tension profile through cornea thickness. Red color is the fit to Bonanno data proposed by Chhabra et al.[2] Black color corresponds to our best fit to same data. Symbols are from Bonanno et al.[1], when the system is composed of cornea+Polymacon2 lens.

DISCUSSION

We have fitted experimental *in vivo* pos-tlens tear-film oxygen tension data as a function of time at tear-film temperature (35ºC), on wearing Polymacon1 and Polymacon2 lenses from Bonanno et al.[1] The only difference between Polymacon1 and Polymacon2 is the thickness, which are 60 μm and 200 μm, respectively. From the Figures 2 and 3, we can see that our best fits are at least similar to the fits of Chhabra et al.[2] And, as can be seen from the values given in Table 1, the only parameter which differs from adjust performed by them is exclusively the value of the corneal oxygen permeability, which in our case we kept constant and approximately equal to that of the water oxygen permeability (93 Barrer), for all systems cornea-lens analyzed.

On the other hand, Figure 3 shows the fit of Bonanno's data with the same parameters obtained by Chhabra et al.[2] for Polymacon2-lens ($Q_{c,max}$=0.9x10^{-4} mL(STp)$\cdot$cm$^{-3}\cdot$s^{-1}; $(Dk)_c$=90 Barrer and K_m=2.2 mmHg), but here with a thickness of

200 μm. However, it can be seen that our parameters (using the same value of the corneal oxygen permeability that in the other systems cornea-lens), allowed us to obtain a good fitting to the experimental data, taking the values of the parameters, K_m and $Q_{c,max}$ in the case of polymacon of 200 μm of thickness, $Q_{c,max} = 0.7x10^{-4}$ (mL(STp)$^{\cdot}$cm$^{-3\cdot}$s^{-1}), $(Dk)_c$=93 Barrer and K_m=2.2 mmHg.

As can be seen, the metabolic model (Michaelis-Menton model), with our parameters successfully reproduces experimental results for transient oxygen tension during closed-eyes contact lens wear and steady state oxygen tension over several lens transmissibilities. The values of our parameters, while fitting the data by Bonanno, provides good results, and the best fits are obtained for Monod dissociation equilibrium constant K_m, and corneal oxygen permeability constant for all systems analyzed. Thus, for a given lens on the cornea, our results reproduce individual experiments in an acceptable manner, maintaining constant the values of the parameters K_m and $(Dk)_c$. However, the maximum oxygen consumption rate diminishes when the oxygen tension at the interface cornea-lens diminish, contrary to what was expected (see Table 2). As occurs in other models, these results may be subject to certain limitations, like the uncertainties in experimental data, especially at high oxygen tensions and this could constitute as an intrinsic limitation of the model itself.

Considering the limitations of the model to explain the rate of change of the experimental data, which does not correspond to the tendency of the value reported by Bonanno et al.[1] at moderate and high pressures ($p \approx 100$ mmHg), where the maximum oxygen consumption rate should be constant independent of the lens wear onto the cornea, it is suggested the occurrence of a kinetic transition that should be assumed as continuous. This kinetics transition can be understood as a consequence of the existence of other effects into the cornea than those referred in the metabolic reactions that occur in the Krebs cycle. Bear in mind that, in the range from low to moderate other phenomena such as corneal swelling can occur. It should be noted that, when it has many parameters in an analysis of experimental data the physical meaning of the values obtained must be taken into account with caution. Particularly, in Chhabra et al, the value of the permeability to oxygen in cornea gives rise to an inappropiate value, which is remedied in our case when this value is given and consequently reducing the fitting parameters.

CONCLUSSIONS

In this paper we present a procedure for solving the non-linear partial differential equation for the position and time depending pressure $p_c(x,t)$, for the oxygen diffusion model of the human cornea, which is an alternative solution respect to Chhabra´s work. In this sense, the novelty of the results obtained here, consists in provide, previous to the solution of the model, the values of diffusion coeffcient Dc and solubility kc. Therefore, the only fitted value is the corneal oxygen-consumption rate $Q_{c,max}$. Despite this limitation the present work shows a revision of the procedure described before by Chhabra et al. 2009, using data previously obtained by Bonanno et al. 2002, to determine the parameters K_m and $Q_{c,max}$ by mean of the Monod kinetics model of oxygen difussion.

As can be seen, the metabolic model (Michaelis-Menton model), with our parameters, successfully reproduces experimental results for transient oxygen tension during closed-eyes contact lens wear and steady state oxygen tension over several lens transmissibilities. Our results reproduce individual experiments in an acceptable manner, maintaining constant the values of the parameters K_m and $(Dk)_c$. Moreover our main finding is that the maximum oxygen consumption rate is not a constant, but diminishes when the oxygen tension at the interface cornea-lens diminish.

Acknowledgement

We thank to Ministerio de Educación y Ciencia (MEC) of Spain for financial support through project ENE2011-24761. LFdC is grateful to SEP-CONACYT for financial support through grant 154626 and project UNAM-DGAPA IN-102512. SIH is grateful to Red Temática de la Materia Condensada Blanda-CONACYT for a postdoctoral fellowship.

APPENDIX

The general equation describing oxygen transport through the lens-corneal system, in one dimension, is Fick's second law with a reaction term,

$$k(x)\frac{\partial P(t,x)}{\partial t} = k(x)D(x)\frac{\partial^2 P(t,x)}{\partial x^2} - Q(P(t,x)) \quad \text{(Eq. A)},$$

where p_c is the oxygen partial pressure in the lens-cornea system, t is time and x is the coordinate normal to the cornea, with x=0 at the interface between the anterior chamber and the cornea.

The second term on the right hand side in Eq. A is the oxygen consumption as a function of the partial pressure, which is absent in the contact lens region and follows a Monod kinetics form in the corneal system:

$$Q(P) = \frac{Q^{max} P}{K_m + P} \quad \text{(Eq. B)}$$

In Eq. A, the solubility (k) and diffusion coefficient (D) are considered function of the position, taking constant values across each of the two regions (contact lens and cornea) in the system. Using the above approach we could obtain the complete pressure profile, provided the continuity of the pressure is satisfied in the lens-cornea interface. This is automatically satisfied within our numerical scheme.

We choose standard Dirichlet boundary conditions in the spatial coordinate:

$P(t,0) = P_{ac}$= 24 mmHg and $P(t, x=L_c+L) = P_{air}$ = 155 mmHg. (Eq. C)

where P_{air} is the open eye pressure, corresponding to the atmospheric pressure, and P_{ac} is the oxygen pressure in the anterior chamber (aqueous humor).

As for the initial condition, in order to reproduce the evolution of the pressure profile from the closed eye condition, we need to feed the stationary pressure profile in Eq. A. This stationary closed eye profile can be obtained by solving the steady-state equation:

$$k(x)D(x)\frac{\partial^2 P_{est}(x)}{\partial x^2} - Q(P_{est}(x)) = 0 \quad \text{(Eq. D)}$$

which is obtained from Eq. A, by removing the temporal evolution. Eq. D is subject to the boundary conditions:

$$P_{est}(0) = P_{ac} \text{ and } P_{est}(x = L_c + L) = P_{PC} \quad \text{(Eq. E)},$$

where P_{PC} is the contact-lens/palpebral conjunctiva oxygen pressure, equivalent to 61.5 mmHg, similar to data used by Chhabra et al.

We then use the solution to Eq. D-E to define:

$$P(0,x) = P_{est}(x) \qquad \text{(Eq. F)}$$

as the last boundary condition for Eq. A.

The system of Eq. D-E and Eq. A-C and F are solved using FiPy (Python Software Foundation), a finite volume PDE solver using Python. Table I shows the different values for the parameters used in the numerical solution of the equations. We use a spatial grid with 10^3 points in all computations, and time steps of 10^{-1}s for the time-dependent equations.

The Eqs. D-E are solved numerically, and the resulting profile is used as initial condition for Eqs. A-C and F. An iterative procedure was used due to the nonlinear nature of the transport equations A to F, by "sweeping" the solutions over few iterations (see FiPy manual for details). Convergence was reached after the residual was below a predefined value (10^{-11} in our case). We checked both, grid size and time step parameters, so that further decrease in size did not result in any improvement. All the computations were performed in a personal computer with an Intel Core i7-3770K under Debian Linux. FiPy version 3.0 was used in all computations.

Multidimensional parameter optimization subject to bounds was done through the "fmin_tnc" function in the Scipy package, which uses a Newton Conjugate-Gradient method. We used this optimization procedure to determine optimized values of the $Q_{c,max}$ and K_m parameters, for a predefined set of the remaining parameters in the model.

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