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Reaction-Diffusion model as framework for understanding the role of riboflavin in "eye defence" formulations

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Analysis of UV-visible spectra, performed on commercial riboflavin-based eye drops, showed that absorbance is a saturating function of vitamin concentration. This means that there is a threshold concentration, C_t, such that for riboflavin concentration > C_t the absorbance remains constant and the effectiveness of the eye drops is independent of the dose used. These experimental results were combined with a diffusion-reaction model to elucidate the mechanism of action within the cornea. The model predicts that the eye drops have a low effectiveness on UVB and UVC, while it has a good performance for UVA. Indeed, at the center of the cornea the transmittance is significantly reduced and after 1 h it is reduced by about 70% compared to a cornea devoid of eye drops.

1 Introduction

Ultraviolet (UV) radiation is the part of electromagnetic spectrum located between X-radiation and visible light. Based on physiological effect it is divided into three primary regions. UV-A $315-400$ nm, UV-B $315-280$ nm and UV-C $280-100$ nm. UV radiation in C region is very dangerous for human health, fortunately is shielded by the atmospheric ozone and does not reach earth's surface. UV-B region make up about 5% of the total UV-radiation, it is mainly absorbed by epidermic cells [1]. UV-A region accounts for 95% of UV rays and it is constantly present throughout the year and over the course of day. The ability of UV rays to penetrate biological tissues increases with decreasing wavelength. A prolonged exposure of the eyes to UV radiation may cause acute, or chronic, effect on the cornea, lens and retina. The World Health Organization estimates that about 20% of the $12-15$ million people who every year risk blindness is caused by sun exposure. Most of UV radiation incident on the eyes is absorbed by cornea and lens, while only 4% reaches the retina. Awareness of UV radiation damage to the eyes has risen substantially over recent years, this has encouraged pharmaceutical companies to look for formulations protecting stressed ocular tissues. Due their easy use, eye drops are generally the preferred means to apply medications when treating ocular disorders.

It is well-known that aqueous solutions of Riboflavin (RBF) (vitamin B_2) protects the eye against UV radiation. However, the outermost epithelium, an hydrophobic layer of $50-100$ μm, is impermeable to hydrophilic drugs, so that 90% of the instilled dose is lost almost immediately. In order to increase the corneal permeability, compounds such as hyaluronic acid, D-α-Tocopheryl polyethylene glycol 1000 succinate, L-proline, methyl sulfonyl metane and many other additives are added to increase the formulation hydrophobicity. The resulting chemical complexity of the formulation makes difficult to understand the mechanism of action of riboflavin in corneal tissue. In this paper absorption spectra of a commercial eye drop are used to elaborate a diffusion-reaction model to evaluate the degree of UVA UVB absorption RBF. Although the model is applied to corneal tissue, it is mathematically presented in a dimensionless form to be extended to other types of RBF administrations. In this paper a diffusion-reaction model to evaluate the efficacy of RBF in commercial eye drops is discussed. The model is presented in dimensionless form to be applied for different administrations, although in the paper it is applied for the protection of the corneal tissue.

2 Theoretical Aspects

2.1 Diffusion model

Initially, the drug exists wholly in a liquid solution at concentration C_0. When a drop of this solution is posed on the biological tissue, as in the *in-vivo* case, tissue becomes wetted, triggering a liquid-solid mass transfer process, providing a means for the drug diffusion in the tissue.

At time $t = 0$ a volume V (typically $0.05 cm^3$) of *Drop Defence* containing N molecules of RBF is placed on the cornea, so that the solution exterior to the organ is C_0. For the sake of simplicity,

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the external solution is assumed to be uniform, *ie* there are no concentration gradients in bulk solution. Therefor, if D is the diffusion coefficient of RBF in the cornea, a simple rate balance over an element in the cornea gives

$$\frac{\partial C}{\partial t} = D\nabla^2 C + r(C) \tag{1}$$

where t is the time, ∇^2 is the Laplace's symbol and $r(C)$ is the reaction term and, generally, is an empirical expression.
In order to solve Eq.(1), the system geometry, initial and boundary conditions have to be established. The problem to be considered herein is that of infinite cylinders in which the diffusion in the radial direction may be neglected. Furthermore it is assumed that initially the concentration of eye drops in the corneal tissue is null. We notice that if oxygen is present in the tissue, the oxidative degradation of RBF can take place. This reaction was investigated under different experimental conditions and was found to be a pseudo first order kinetics. Therefore the molecular concentration of RBF, at position x and time t assume the form

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - KC \tag{2}$$

where K is a pseudo first-order rate constant. The initial and boundary condition are

$$\begin{cases} C(x,0) = 0 \\ C(0,t) = C_0 \\ \lim\limits_{x\to\infty} \frac{\partial C}{\partial x} = 0 \end{cases} \tag{3}$$

Eq.2 may be solved by the Laplace transform with ease. Details of this procedure will be omitted and the solution will merely be sketched.

$$\frac{C(x,t)}{C_0} = \frac{1}{2}\left[e^{-x\sqrt{\frac{K}{D}}} erfc\left(\frac{x}{\sqrt{4Dt}} - \sqrt{Kt}\right) + e^{x\sqrt{\frac{K}{D}}} erfc\left(\frac{x}{\sqrt{4Dt}} + \sqrt{Kt}\right)\right] \tag{4}$$

The process involves diffusion and chemical reaction in a tissue, depending on the relative rates of these individual processes, one of the them would control the overall kinetics. Specifically diffusion will control if reaction rate is fast. To delineate these regions, it is useful to define different lifetimes[2]. Thus, we define $t_D = \mathscr{L}^2/D$ as the diffusion lifetime and $t_R = 1/K$ as the reaction lifetime, $\mathscr{L}$ being a characteristic system length. To convert Eq.4 to non-dimensional form, we adopt as unit of length $\mathscr{L}$ and an associated unit of time Now, we re-scale the lengths with $\mathscr{L}$ and the times with t_D, *ie* we set $X = x/\mathscr{L}$ and $\bar{t} = t/t_D = t\mathscr{L}^2/D$, so that Eq.4 is transformed into

$$\frac{C(x,t)}{C_0} = \frac{1}{2}\left[e^{-pX} erfc\left(\frac{x}{\sqrt{4\bar{t}}} - p\sqrt{\bar{t}}\right) + e^{pX} erfc\left(\frac{x}{\sqrt{4\bar{t}}} + p\sqrt{\bar{t}}\right)\right] \tag{5}$$

where $p = \sqrt{t_D/t_R}$ it is the only external parameter to be determined to calculate the generalized concentration profile.

2.2 Electromagnetic-radiation absorption

An electromagnetic wave incident on a material undergoes an attenuation in its intensity due to absorption. The latter phenomenon is described by absorption coefficient, σ_t, defined as the ratio between the intensity absorbed in the unit volume and the intensity incident per unit area. With reference to a light beam propagating along x direction, the fraction of intensity lost in the volume element, dV, is, according to the definition, $\sigma_t dV \cdot (\frac{I}{\Sigma})$, where Σ is the cross section of the beam and I the impinging intensity. The variation of intensity passing through the volume element, therefore, is

$$-dI = \sigma_t dV \cdot \frac{I}{\Sigma} = \sigma_t I dx \tag{6}$$

where $dV = \Sigma dx$ has been used.
Integrating Eq.6, Lambert-Beer law is obtained

$$\frac{I}{I_0} = exp\left(-\int_0^x \sigma_t dx'\right) \tag{7}$$

In Eq.7 the coefficient σ_t takes into account the overall attenuation suffered by the wave, in the considered volume. However, if the volume is composed of two or more phases each individual contributes has to be explained. In our model, phases consist of a liquid solution (eye drops) and a solid phase (cornea) so that absorption coefficient assumes the form

$$\sigma_t = \sigma^l(\lambda, C) + \sigma^s(\lambda) \tag{8}$$

where σ^l and σ^s are liquid and solid contributes, respectively. In Eq.8 is highlighted that the absorption coefficient depends both on the wavelength of the incident light and on the the molecular composition of the material. In the solid phase, at a fixed wavelength, σ^s is constant and represents the inverse of the penetration depth, δ_p in the material. Although σ^s can be estimated using electromagnetic field equations in dielectrics, we preferred to calculate it directly from experimental presented in the literature. In order to compute $\sigma^l(\lambda, C)$, we note that the absorbent concentration is always extremely small so that, at a fixed wavelength, the first term of a Taylor series of function σ provides an accurate value. Thus, we can write

$$\sigma_t = \bar{\sigma}(\lambda)C + \frac{1}{\delta_p} \tag{9}$$

where

$$\bar{\sigma}(\lambda) = \left(\frac{\partial \sigma^l}{\partial C}\right)_{C=0} \tag{10}$$

combining Eq.8 and Eq.9 we have

$$\frac{I}{I_0} = exp\left(-\int_0^x \bar{\sigma}(\lambda)C dx' - \frac{x}{\delta_p}\right) \tag{11}$$

Based on Eq.10 definition, the term $\bar{\sigma}$ should be concentration-independent, however the system non-ideality is reflected on this parameter in a complex way so that results constant only for very small concentration intervals. This implies that the integral can only be solved if one knows the functional dependence $\bar{\sigma}(C)$. Such dependence can only be deduced by experimentation. Bearing in mind that the absorbance is defined as $\mathscr{A} = -log_{10}(\frac{I}{I_o})$, it

is convenient to rewrite Eq.11 in the form

$$\frac{I}{I_0} = e^{-X\frac{\mathscr{L}}{\delta_p}} exp\left(-\frac{\mathscr{L}}{l}\int_0^X \mathscr{A}_{exp}(\lambda,C)dX'\right) \qquad (12)$$

where

$$\mathscr{A}_{exp}(\lambda,C) = \varepsilon(\lambda,C)Cl \quad \text{with} \quad \varepsilon = \bar{\sigma} log_{10} e$$

is the spectrophotometrically measured absorbance. The term $\mathscr{L}/l$ appears because in experimental practice l is measured in cm while length $\mathscr{L}$ is arbitrary, in other words it takes into account the different measurement units used for light absorbance and molecular diffusion.

3 Materials and methods

3.1 Chemicals

Ophthalmic antioxidant formulation, termed *drop defence*, was kindly offered by Iros sc S.r.l. (Italy), patent no. EP 2459186, USP 9192594). According to the manufacturer drop defence is a mixture of riboflavin (RBF), d-α-tocopheryl polyethylene glycol (TPGS vitamin E), proline, glycine, lysine and leucine solution, pH 7.2-7.4. Phosphate buffered saline (PBS) solution was prepared dissolving one tablet, purchased by Sigma Aldrich, into 0.2 dm^3.

3.2 Solutions preparation

Since the detailed composition of the eye drops is patented, the concentration of the individual constituents was varied by diluting the commercial sample by means of PBS solutions PBS buffer (10 mM, pH 7.2). In order to reduce volume errors due to dilution, all solutions were always prepared by dilution of the eye drops and not by subsequent dilutions. This means that if C_0 is the initial concentration of any solution constituent, after dilution the concentration turns out to be

$$C = \alpha C_0 \qquad (13)$$

α being the dilution factor.

3.3 Methods

3.3.1 Spectrophotometry

The absorption measurements were carried out with a cary 100-Varian UV-Vis equipped with thermostatted cuvettes. Samples were placed in a rectangular quartz cuvettes of 1 cm path length and absorption spectra were recorded at 25±0.5)°C in the 200-800 nm wavelength region.

4 Results and Discussion

4.1 Absorption spectra

Figure1 shows absorption spectra of the commercial eye drops measured at 25°C as a function of the dilution factor C/C_0. As one can see, each spectrum is characterized by one peak in visible light range at 445 nm and three peaks in UV range at 220, 266, 373 nm. Absorption of light in the UV-visible region is due to electronic transitions between ground state and excited state valence

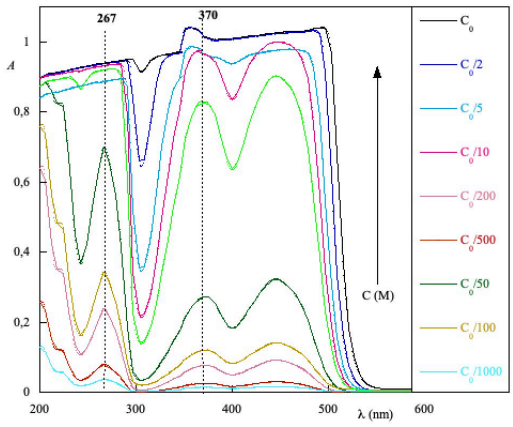

Fig. 1 Absorption spectra of commercial eye drops as a function of the dilution factor

electrons. Eye drops RBF-based, absorb visible light due to the network of conjugated double bonds in the molecule. However, spectra in Fig. 1 are complex because the in RBF molecules superposition of rotational and vibrational transitions with the electronic transitions gives a jumble of overlapping lines that appears as a continuous absorption band. Comparing the wavelengths of experimental peaks with the physiological ranges of UV radiation, one realizes that $\lambda =$ 371 nm falls in the UVA range while other two wavelengths lie in the UVC range. Therefore, the effect of RBF concentration on UV radiation was studied by monitoring abosorbance peaks at 370 and 260 nm as well as the absorbance value at 300 nm, as a function of the dilution factor. Results are displayed in Fig.2. An detailed numerical analysis indicates that

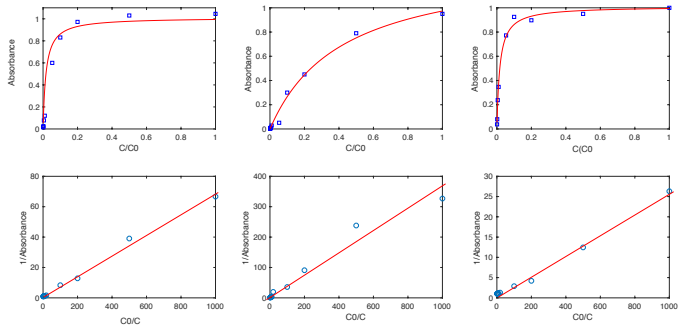

Fig. 2 Absorbance at

data displayed in well-described by a saturation curve of the type:

$$\mathscr{A}_{exp} = \mathscr{A}_{max}\frac{m_\lambda \alpha}{1+m_\lambda \alpha} \qquad (14)$$

To be sure of eq.15 a further test was performed by plotting, for all the wavelengths investigated, reciprocal of $\mathscr{A}_{exp}$ vs. reciprocal of α. Fig.2 highlights, for all the cases, an excellent linearity, assuring us that eq.15 is a good representation of the experimental data. Parameters for each curve were computed by means of nonlinear and are collected in tab1. We notice that $\mathscr{A}_max$ is independent of λ, with a constant value around 1, on the contrary m_λ is strongly dependent on the wavelength. This observation im-

plies that experimental curves can be substantially described by a single parameter, for any RBF concentration. In order to convert eq.15 to a more practically useful form, we make use of eq.3 and obtain

$$\mathscr{A}_{exp} = \mathscr{A}_{max}\frac{C}{C_t + C} \tag{15}$$

where

$$C_t = C_0/m_\lambda \tag{16}$$

In this form it is immediately seen that there exist a threshold concentration which determines two regimes. Indeed, for $C < C_t$, experimental function $\mathscr{A}_{exp}$ increases linearly with RBF; for $C > C_t$ the function $\mathscr{A}_{exp}$ is practically constant. Likely, at high concentrations the riboflavin molecules associate in dimers, trimers, n-mers forms, making the electrons less available to pass in excited states, accordingly experienced absorbance remains constant. Finally for $C = C_t$, $\mathscr{A}_{exp} = \mathscr{A}_{max}/2$. The mathematical form of the absorbance obtained experimentally has some direct consequences for actual utilization and formulation of the eye drops. Indeed, by definition threshold concentration depends on λ through m_λ and on C_0. This means that C_t can be suitably modulated during the preparation of the formulation. At first glance, therefore, one might be tempted to conclude that, for this formulation, at high C_0, the eye drops should give an excellent result UV absorption. A second look of Fig 3, however, shows that this conclusion is not justified because from C_t onwards the absorbance is constant, a large excess of RBF relative to Ct implies a large amount of unused substance. If, then, we consider that RBF undergoes oxidative degradation with formation of free radicals, one realizes that the RBF quantity to be used must be calibrated so as to have low C_t and low probability for the oxidation reaction

Table 1 Experimental results

λ (nm)	$\mathscr{A}_{max}$	m_λ
267	1.1±0.1	21±3
370	1.0±0.2	58± 6
300	1.3±0.5	2.9±0.2

4.2 Application

Eq.15 gives the experimental absorbance in terms of RBF concentration which is a function of independent normalized variables X and $\bar{t}$, as established by Eq.5. The substitution of both these equations into Eq.(12) enable us to compute the transmittance in a tissue as a function of the position and time. Obviously, in order to evaluate such a function within the cornea, the diffusive and photo-dissociative behavior of RBF in the organ has to be known. The diffusion coefficient of RBF in the cornea, $D = 6.5 \cdot 10^{-7}$ cm^{2}s^{-1}, was obtained by Quaid et al.[3]. The value of $\tau_R = 0.91 \cdot 10^5$ s for photo-dissociation of RBF molecules was interpolated from data summarized by Ahmad et al.[4]. From these data the control parameter $p = 0.23$ is immediately derived and the RBF concentration, as a function of X and $\bar{t}$, calculated. However, in order to numerically determine the total transmittance of UV radiation it is necessary to know their penetration length in cornea. From results reported by Kolozsá et al.[5] and Čejka et al.[6], we calculated $\delta_p = 0.19$ cm, for $\lambda = 373$ nm; $\delta_p = 0.0090$ cm, for $\lambda = 300$ nm; and $\delta_p = 0.0269$ cm, for $\lambda = 266$ nm. Withe these parameters and results of Tab.1, the integral in Eq.12 is numerically computed and the function $I(X,\bar{t})/I_0$ is determined, for each fixed wavelength. Results collected as 3D-plots in Fig. 3 indicate that UVB radiation

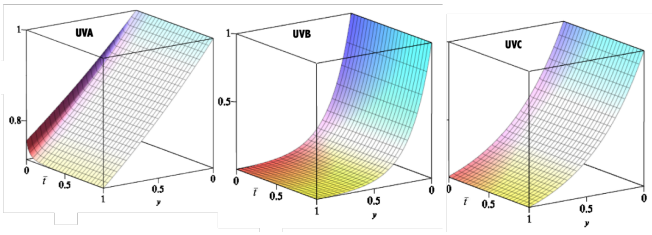

Fig. 3 Absorbance at

is rapidly attenuated and completely absorbed within $X = 0.4$, ie 40% of cornea length. UVC radiation is less rapidly attenuated, but anyhow it is absorbed within $X = 0.90$, that is to say 90% of cornea length. These results follow from the fact that, for these wavelengths, δ_p is very small so that the pre-exponential term in Eq.12 causes a very strong damping, regardless of the integral value. In other words, the intrinsic chemical composition of the cornea protects it against UVB and UVC radiation. Accordingly, RBF-based eye drops have a negligibly small effectiveness in this UV range. Regarding UVA radiation, Fig.3 shows that the attenuation occurs along the whole cornea and absorption is significantly dependent on the RBF concentration.

The above discussion is general and may always be applied to determine the absorptive properties of the system under investigation. However, it is not practical to do so because the necessary integrations require that an analysis of 3D plots be available. A more useful and alternative equation is needed for predictive calculation of absorbance in the cornea. Such equation is obtained by noting that C_t identifies two regimes with different absorptive characteristics.

Regime $C(X,\bar{t}) > C_t$.

In this case Eq.15 is simplified in

$$\mathscr{A}_{exp} = \mathscr{A}_{max} C(X,\bar{t}) \tag{17}$$

so that Eq.15 is analytically solved and results in

$$\frac{I(y,t)}{I_0} = e^{-\frac{\mathscr{L}}{\delta_p}y} e^{-sG(X,\bar{t})} \tag{18}$$

with

$$s = \frac{\mathscr{L}}{l}\ln(10)\frac{\mathscr{A}_{max}}{p} m_\lambda \tag{19}$$

and

$$G(X,\bar{t}) = \frac{e^{py}\mathrm{erfc}(\frac{y}{\sqrt{4\bar{t}}} + p\sqrt{\bar{t}}) - e^{-py}\mathrm{erfc}(\frac{y}{\sqrt{4\bar{t}}} - p\sqrt{\bar{t}})}{2} + \mathrm{erf}(p\sqrt{\bar{t}}) \tag{20}$$

In Eq.18 the first exponential is the cornea contribution to UVA attenuation, the second one is due to eye drops. This latter depends both on the molecular properties of RBF and on its spatio-

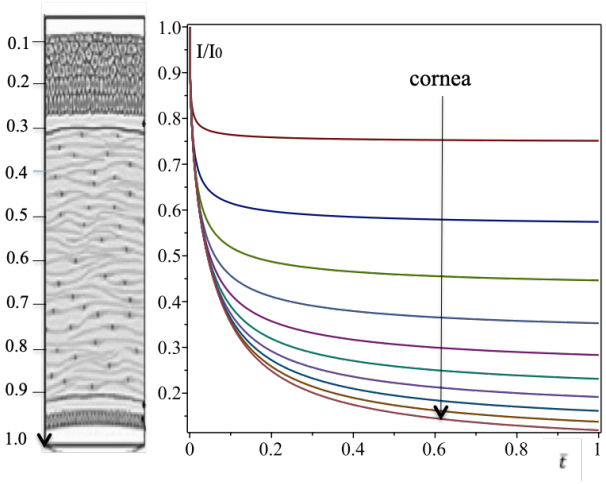

Fig. 4 Absorbance at

temporal evolution. Therefore, such contribution was analyzed separately for fixed values of X and $\bar{t}$. To calculate the transmit-

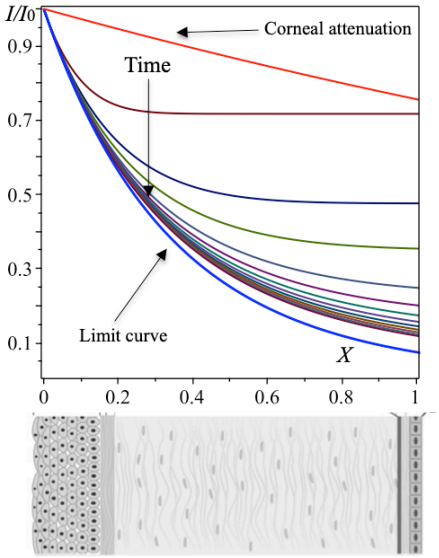

Fig. 5 Absorbance at

tance as a function of time, we first divide X-axis into region $[0,1]$. Then $X_n = n/10$ defines points which span the $X-$axis contained within the cornea. The next step consists of applying Eq.18 for each fixed X_n; results are illustrated in Fig.4. As one can see, I/I_0, for each n, decreases to a plateau, point where the attenuation is saturated. We note that for $\tau_D/\tau_R = p^2 \ll 1$ photo-dissociation reaction is slow compared with diffusion. This means that RBF concentration inside the cornea is governed by on molecular diffusion. For example, with aid of Fig.4, one finds that for $n = 1$ the attenuation saturates in 7 min, while for $n = 0.5$ after 30 min.

To calculate the transmittance as a function of variable X, the normalized time $\bar{t}$ is probed at intervals of 0.1. Results displayed into Fig.5 indicate that also in this case the attenuation saturates to large distances X. Interestingly, for long times all the attenuation curves converge in a limit curve (blue curve in Fig.5). This numerical observation is analytically verified, indeed

$$\lim_{\bar{t}\to\infty} G(X,\bar{t}) = 1 - e^{-pX} \tag{21}$$

In Fig.5, the attenuation of the cornea without eye drops is also plotted for comparison (red curve). Careful analysis of the plot makes it clear that eye drops significantly reduce transmittance. Indeed, at the center of the cornea, in the absence of eye drops, $I/I_0 = 0.85$, while in the presence, after 1.2 h the transmittance becomes 0.21, with a net gain of 75%.

5 Conclusions

UVA and UVB exert different effects on biological tissue, determined by their respective wavelengths. RBF based eye drops are generally used to protect the eyes from UV radiation. Nevertheless, RBF is subject to photodegradation reactions which reduce its effectiveness. The effects of a commercial RBF-based eye drops have been studied by means of a diffusion-reaction model.

Conflicts of interest

There are no conflicts to declare

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