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Measuring the viscosity of whole bovine lens using a fiber optic oxygen sensing system

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Purpose: To obtain a better understanding of oxygen and nutrient transport within the lens, the viscosity of whole lenses was investigated using a fiber optic oxygen sensor (optode). The diffusion coefficient of oxygen was calculated using the Stokes-Einstein equation at the slip boundary condition.

Methods: The optode was used to measure the oxygen decay signal in samples consisting of different glycerol/water solutions with known viscosities. The oxygen decay signal was fitted to a double exponential decay rate equation, and the lifetimes (tau) were calculated. It was determined that the tau-viscosity relationship is linear, which served as the standard curve. The same procedure was applied to fresh bovine lenses, and the unknown viscosity of the bovine lens was calculated from the tau-viscosity relationship.

Results: The average viscosity in a whole bovine lens was determined to be 5.74±0.88 cP by our method. Using the Stokes-Einstein equation at the slip boundary condition, the diffusion coefficient for oxygen was calculated to be 8.2 × 10^{-6} cm^2/s.

Conclusions: These data indicate a higher resistance to flow for oxygen and nutrients in the lens than what is currently assumed in the literature. Overall, this study allows a better understanding of oxygen transport within the lens.

Cataract is the leading cause of blindness affecting 20 million people worldwide [1]. It is a multifactorial ocular disease caused by genetics, age, and environment. These environmental factors include hyperbaric oxygen treatments and vitrectomy, which are directly related to high oxygen exposure [2,3]. There are reports in the literature that oxidative stress damages the structure of the lens by altering the lens fiber cells and causing aggregation of the crystallin proteins [4,5]. These data highlight the significance of maintaining low oxygen levels in the lens.

An understanding of oxygen transport in the lens is important to elucidate the mechanisms that lead to cataract via oxidative stress. The lens is an avascular tissue that allows oxygen to diffuse inward from adjacent compartments [6]. Within the lens, an oxygen gradient is further observed that leaves the lens nucleus to contain virtually no oxygen [3,7]. The low oxygen level within the lens is maintained by the lipid membranes and mitochondria in the lens cortex by consuming oxygen that is diffusing inward at a slow rate [8,9]. The transport of oxygen has been assumed to have no resistance to flow, although there are no data to support this.

The Stokes-Einstein equation at the slip boundary condition can be used to calculate the diffusion coefficient of oxygen if the viscosity of the medium is known. This is an approximation since the oxygen molecules are smaller than the lens proteins. Currently, two reports in the literature address lens viscosity. Tiffany and Koretz determined the viscosity of α-crystallin solutions at different concentrations and temperatures. At physiologically relevant concentrations from 250 to 300 mg/ml of α-crystallin, the range is approximately 3–7 cP [10]. This value differs from other values used in the literature such as by Dierks et al. (1998). In their study, they assumed the viscosity is 2 cP after determining the viscosity of different concentrations of lysozyme samples [11]. There are no reports about the viscosity of a whole lens to date. Since the lens is a heterogeneous mixture, traditional methods cannot be used to measure the viscosity.

Therefore, the purpose of this study is to investigate the physical properties of whole lenses using a fiber optic oxygen-sensing probe (optode). The novel method was used to measure the viscosity of bovine lenses, which have protein and lipid profiles comparable to young human lenses [12-14]. These data will give an insight into the transport of oxygen and nutrients within the lens.

METHODS

Glycerol/water solutions: Only the highest-quality chemicals available were used in this study. Glycerol was purchased from Sigma Chemical (St. Louis, MO). Water was purified using a Millipore Milli-Q-Plus Purepak 2 water purification system (EMD Millipore, Bellerica, MA). The glycerol

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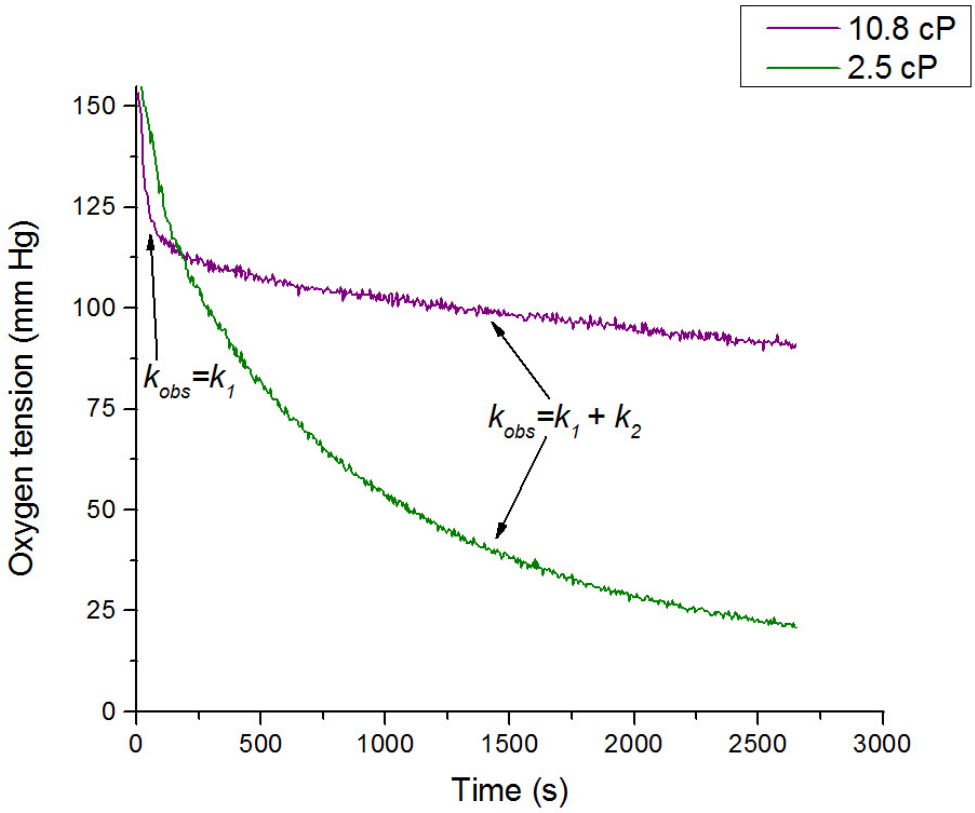

Figure 1. The comparison of decay signals between two different glycerol/water solutions. Low viscous glycerol/water solutions such as 2.5 cP quickly reach equilibrium so the curve resembles one-phase decay. Higher viscosity glycerol/water solutions exhibit distinctive two-phase decay as demonstrated with the 10.8 cP solution.

solutions were prepared with 0%, 10%, 20%, 30%, 40%, 50%, 60%, and 70% (w/v) of glycerol/water with known viscosity of 1.0–22.5 cP at 25 °C as reported by Segur and Oberstar [15]. The samples were bubbled with argon for 30 min, the minimum time required to remove oxygen from the samples.

Lenses: Seventeen bovine eyes were obtained from the local abattoir (Aurora Packing Company, Aurora, IL). These tissues were all excised and from animals that were less than 30 months old. The tissues were obtained 4–5 h after slaughter and stored at 4 °C for up to 30 h. Each lens was isolated by making a scleral incision to remove the compartments of the anterior segment and cutting the vitreous gel. The lens was immediately weighed. Then the lens was placed in a container and bubbled with argon for 30 min to remove oxygen from the container. The lens was ready for measurement after no signs of cloudiness were observed.

Measurement of viscosity: All measurements were performed with the fiber optic oxygen sensor system (Ocean Optics, Dunedin, FL). The system consists of the FOXY-AL-300-TM probe (optode), which is 500 µm in diameter with an aluminum jacket. This spectrometer-coupled chemical sensor analyzes dissolved and gaseous oxygen pressure. The probe tip is coated with a sol-gel material containing a ruthenium complex. When exposed to a light-emitting diode (LED) excitation, the ruthenium compound fluoresces. The presence of oxygen causes a quenching of the fluorescence signal from the ruthenium compound resulting in a phase shift. The difference in phase shift is related to the partial pressure of oxygen and is recorded in the SpectraSuite software. The optode is precalibrated by the manufacturer. Based on the manufacturer's report, the measurement range of dissolved oxygen in liquid is 0–760 mmHg, and its resolution is 0.4 mmHg at room temperature.

The optode was mounted on a micromanipulator directly above the samples. It was air saturated before the tip of the optode was inserted through the anterior surface of the lens and 3 mm into the samples. It was allowed to stay in one place to record until the signal no longer changed. Before the next sample, the optode was air saturated once more.

Analysis: Oxygen initially leaches from the probe followed by an approach to equilibrium between the oxygen in the probe and the oxygen in the surrounding solution. Based on this, we assume the following process is occurring in the probe:

$$Oxygen_{Air} \underset{k2}{\overset{k1}{\rightleftharpoons}} Oxygen_{\sim 0\%}$$

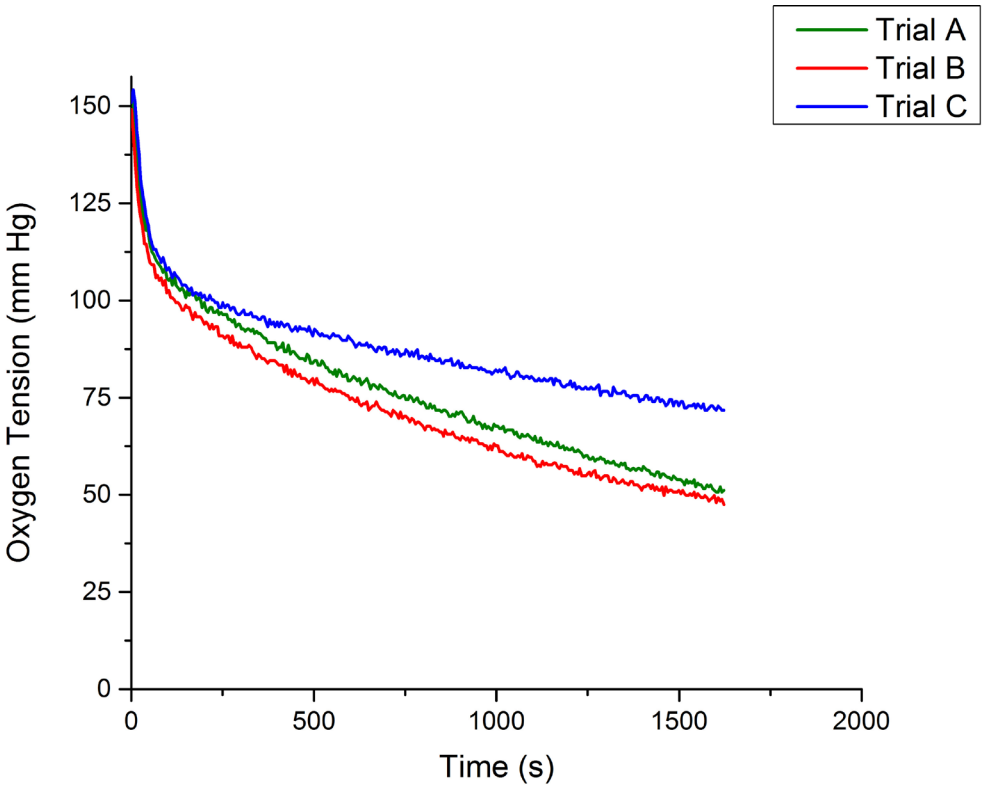

Figure 2. The reproducibility of the oxygen-sensing probe. The signals observed for a 6 cP glycerol/water solution demonstrate that solutions of the same viscosity produce similar decay curves, which are reproducible under controlled experimental conditions.

The longer-lived component may correspond to the approach to equilibrium:

$$k_{obs} = k_1 + k_2$$

In Figure 1, a comparison of signals from two solutions with different viscosity is shown. In samples with a low viscosity (ca. 2.5 cP), the equilibrium was quickly reached. The best fit to the data was a single exponential fit. In higher viscosity samples (ca. 10.8 cP), the initial leaching of oxygen was slower, *kobs=k1*, followed by the equilibrium. In these cases, the data were fitted to a biexponential fit. The lifetime of the slow component (τ) was determined by fitting the decay signal with OriginPro 8.6 software (Northampton, MA).

The Stokes-Einstein equation at the slip boundary condition relates diffusion coefficient (D) to the viscosity (η) by the following equation:

$$D = kT / 4\pi\eta r$$

where *k* is the Boltzmann constant, T the absolute temperature, and r is the radius of the oxygen molecule. This boundary condition is the most appropriate for solute and solvent molecules with similar radii; thus, it is used in calculating self-diffusion coefficients.

RESULTS

Measurements with glycerol/water solutions: Figure 2 shows general optode signals from three solutions with 6 cP. These signals produced similar decay patterns and were reproducible under controlled experiments.

Tau for the slow component was calculated for each glycerol/water mixture. It was determined that tau increased with the viscosity of the solution. Linear regression was performed on the tau versus viscosity plot as shown in Figure 3. The results of this fit are recorded with the following equation at 25 °C which served as the standard curve.

$$y = 167.5x + 490.6$$

Measurements of bovine lenses: Figure 4 shows four typical optode signals from whole bovine lenses. The optode signals were fitted to biexponential decay. Tau was calculated for the slow component. Using the standard curve, the viscosity was determined. The average viscosity is 5.74±0.88 cP with an average mass of 1.98±0.13 g for 17 lenses. Using the Stokes-Einstein equation at the slip limit, the diffusion coefficient of oxygen was calculated to be 8.2×10^{-6} cm^2/s.

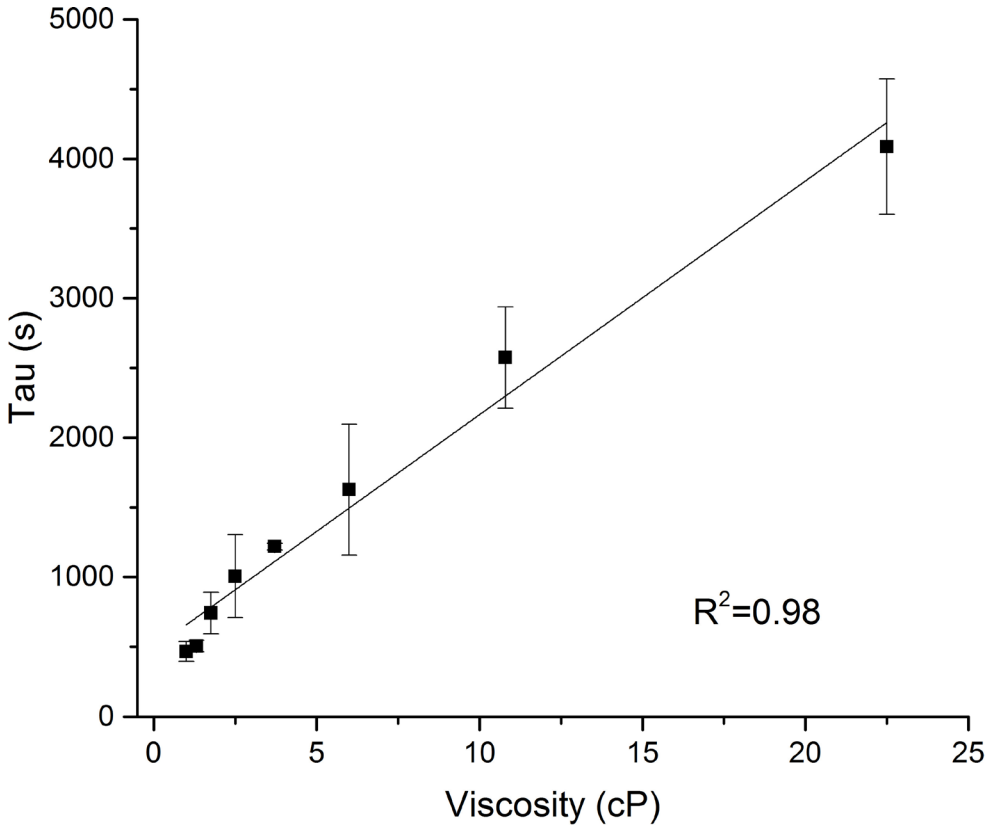

Figure 3. The tau-viscosity relationship. This is a linear fit for the variation of viscosity with exponential decay lifetimes.

DISCUSSION

Since the viscosity of the whole lens is unknown, our study sought to determine it by using an optode. The decay rates of oxygen tension for different glycerol/water concentrations were measured using the optode. Tau of the slow component was calculated for each sample and plotted against the viscosity to create a standard curve. This relationship was used to quantify the viscosity of whole bovine lenses.

The optode senses oxygen tension based on the oxygen quenching of a ruthenium complex. The equilibrium occurs in all solvents; however, the ruthenium complex lifetime increases proportionally with solution viscosity [16]. The trends of our glycerol/water mixtures lifetimes were consistent with these previous findings.

This is the first study to measure the viscosity of whole bovine lens. Nevertheless, a comparison can be made with studies in the literature. The viscosity of 2 cP reported thus far in the literature is lower than our average viscosity [11]. The measurements in that study were based on lysozyme, a 14 kDa protein that is smaller than the native intrinsic lens proteins. Recently, Schachar et al. calculated the viscosity of fresh porcine and human lenses to be 160 cP and 330 cP, respectively [17,18]. In their studies, they used a linear, simple-shear rheometer to measure the complex shear modulus of a 1 mm section of lens. Rheometers are generally designed for homogenous liquid mixtures, but an intact lens is a heterogeneous, complex structure. In these reported experiments, the capsule was removed, and the lens structural integrity may have been compromised. We are not sure why there is such a large discrepancy between our viscosity value and that reported by Schachar et al., but it may be due to the different species.

Another comparison can be made with the viscosity of α-crystallin solutions measured by Tiffany and Koretz (2002). They reported a range of values for the viscosity of α-crystallin solutions at different temperatures. Our average viscosity is consistent with their finding of 3 cP at a typical protein concentration of α-crystallin at 25 °C, which is greater than 3 cP.

Altogether, these findings suggest that the lens viscosity depends on the α-crystallin concentration. This predominant crystallin belongs to a small heat shock protein family and makes up 40% of the total lens proteins [19]. It interacts with all components of the lens including the other crystallins, β and γ [20], as well as membranes [21]. Relatively low levels of αB-crystallin are also found in non-ocular tissues such as

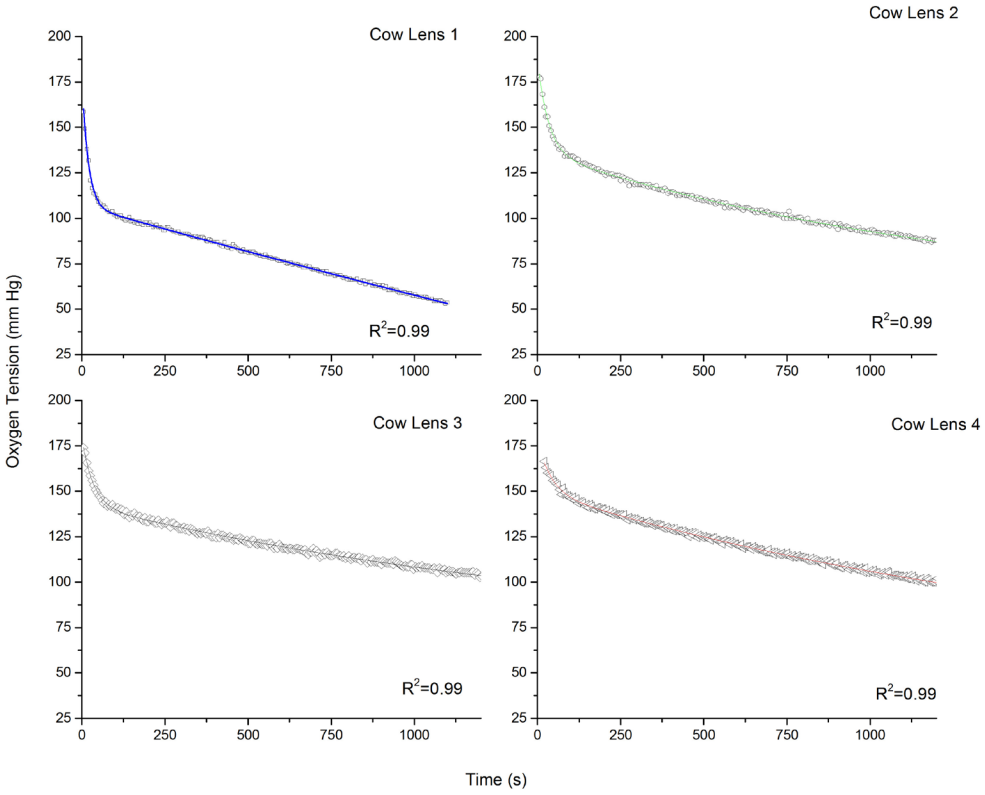

Figure 4. Typical oxygen decay signals from the bovine lenses as represented by four different lenses. All samples exhibited a two-phase decay pattern similar to the high viscosity samples.

the heart, lungs, brain, and spleen [22]. Interestingly, skeletal muscle also expresses αB-crystallin [23] where the oxygen diffusion coefficient in skeletal muscle is 1.3×10^{-5} cm^2/s [24]. This diffusion rate is higher than in the lens. In addition, hemoglobin is a large protein with a molecular mass similar to the crystallin proteins. In a 30% hemoglobin solution, Klug et al. reported the oxygen diffusion coefficient is 7.1×10^{-6} cm^2/s, which is similar to our calculated value [25].

The lens fiber membrane also provides resistance to oxygen transport. The membranes are saturated with high concentrations of phospholipids and cholesterol [26,27]. Raguz et al. have shown that phospholipid and phospholipid-cholesterol mixture have a lower oxygen permeability coefficient when compared to water [28]. More specifically, the cholesterol crystalline domains have the most resistance to oxygen permeation [29,30]. Membranes that are not saturated with phospholipids and/or cholesterol have no resistance to oxygen flow. For example, Fischkoff and Vanderkooi (1975) reported that the oxygen diffusion rate for erythrocyte plasma membrane is 2.2×10^{-5} cm^2/s identical to the diffusion rate in saline. The diffusion rate is similar in lecithin-based membranes with comparable concentrations found in other tissues [31].

In this present study, we measured the bulk viscosity of young, healthy bovine lenses as a proof of concept. We did not differentiate between nuclear and cortical positions. However, several groups reported that the stiffness increased with age, especially in the lens nuclear region [32,33]. Furthermore, the refractive index and Brillouin scattering in the lens nuclear region is different from the cortical region [34,35]. All of these studies suggest that the viscosity may be different between these positions. Therefore, in the future, our method must be refined to apply it to human lenses and differentiate between the nuclear and cortical positions.

In this study, a method was developed to characterize the viscosity of whole bovine lenses with an optode. In the future, these studies will be extended to measure the viscosity of human lenses, both cataractous and non-cataractous. The information will provide a better understanding of alterations in the lens.

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