THE JOURNAL OF PHYSICAL CHEMISTRY B

ARTICLE

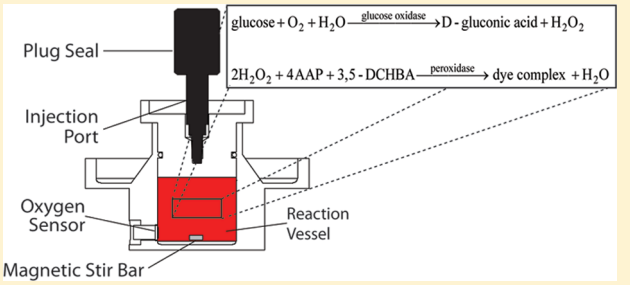
pubs.acs.org/JPCB

Complementary Methods for the Determination of Dissolved Oxygen Content in Perfluorocarbon Emulsions and Other Solutions

Christopher A. Fraker,[†] Armando J. Mendez,[§] and Cherie L. Stabler*[,†,‡]

[†]Department of Biomedical Engineering, [‡]Department of Surgery, and [§]Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, Diabetes Research Institute, Leonard M. Miller School of Medicine, University of Miami, 1450 NW 10th Ave, Miami, Florida 33136, United States

ABSTRACT: Perfluorocarbons (PFCs) are compounds with increased oxygen solubility and effective diffusivity, making them ideal candidates for improving oxygen mass transfer in numerous biological applications. Historically, quantification of the mass transfer characteristics of these liquids has relied on the use of elaborate laboratory equipment and complicated methodologies, such as in-line gas chromatography coupled with temperature-controlled glass fritted diffusion cells. In this work, we present an alternative method for the determination of dissolved oxygen content in PFC emulsions and, by extrapolation, pure PFCs. We implemented a simple stirred oxygen consumption microchamber coupled with an enzymatic reaction for the quantitative determination of oxygen by optical density measurements. Chambers were also custom fitted with lifetime oxygen sensors to permit simultaneous measurement of internal chamber oxygen levels. Analyzing the consumption of oxygen during the enzymatic reaction via recorded oxygen depletion traces, we found a strong degree of correlation between the zero-order reaction rate and the total measured oxygen concentrations, relative to control solutions. The values obtained were in close agreement with published values in the literature, establishing the accuracy of this method. Overall, this method allows for easy, reliable, and reproducible measurements of oxygen content in aqueous solutions, including, but not limited to PFC emulsions.



INTRODUCTION

Perfluorocarbons (PFCs) are chemically inert compounds constructed of straight or cyclic carbon skeletons completely saturated with fluorine, other halides, or combinations thereof. The halogenation of the carbon skeletons of PFCs renders them extremely hydrophobic and nonpolar, a structural characteristic that imparts enhanced gas solubilities and transfer capacities. This is particularly true of carbon dioxide and oxygen, two critical respiratory gases. For these reasons, PFCs and PFC emulsions have been implemented in numerous clinical and research applications. For the last half of a century, substantial research has investigated the use of pure PFCs and micellar PFC suspensions (emulsions) as oxygen carriers.[1−19] Functional studies of various PFCs have found their oxygen solubilities to approximate or surpass hemoglobin, at 12−20 times that of water or physiological salt solutions at varied oxygen partial pressures.[11,14,15,20−25] Additionally, the dissolved oxygen content in some PFCs does not vary substantially with temperature, resulting in further enhancement in oxygen solubility due to the concomitant decreased solubility within physiological solutions.[26] In addition to increased oxygen solubility, the effective oxygen diffusivities of PFCs are approximately 2.5- to 4-fold that of water or physiological salt solutions, such as culture medium. These highly desirable properties have been shown to enhance the oxygen transfer rate (i.e., diffusive permeability) in several studies, where they have been utilized in oxygen delivery capacities as parenteral blood substitutes and in organ preservation solutions.[23,27−30]

The most common implementation of PFCs to enhance oxygen transfer is in the form of nanoscale emulsions/micellar suspensions. Given their hydrophobicity, PFCs are typically suspended in aqueous solutions of amphiphilic surfactants, where high-pressure microfluidization is used to generate stable, micro- or nanoscale emulsions. Given this requirement and their conventional use as intravenous oxygen carriers in medical procedures requiring transfusion, it is critical that they are manufactured in a size range that does not have the potential for causing thrombotic events in patients. A further requirement is emulsion stability, as change in particle size could adversely affect patient safety, as well as emulsion efficacy. Typical formulations of PFC emulsions are composed of polar lipid-based (egg-yolk phospholipids, cholesterol) or PEG-based (Pluronics) surfactants and PFC diluted in a physiological, aqueous phase. While substantial investigation into the individual properties of emulsion components has been conducted, there has been incomplete published characterization of manufactured emulsions in regard to complete assessment of oxygen transfer properties.[31−34] Typically, theoretical estimations based on measurements in pure PFCs are used, which may not translate perfectly to their particular solution. Thus, there is a need to fully

Received: May 4, 2011
Revised: July 26, 2011
Published: July 27, 2011

characterize emulsion parameters including dissolved oxygen capacity, diffusivity, and stability, particularly for use in clinical applications.

In PFC emulsions, the enhanced oxygen mass transfer is the product of the increased effective oxygen diffusivity, D_{eff}, and inherently higher oxygen solubility of PFCs, S_{O_2}, which is defined as diffusive permeability, P_D. Critical to the characterization of the benefit afforded by a particular PFC emulsion is the accurate determination of both of these variables. Historically, solubility has been demonstrated in the literature to have the most substantial effect on the diffusive permeability of PFC emulsions, as the oxygen solubility of pure PFCs is an order of magnitude greater than physiological buffers (13- to 20-fold).[1,11,14,15,23,26,27,35] Therefore, accurate determination of the dissolved oxygen content of PFC emulsions, which are composed of hydrophilic and hydrophobic phases and varying PFC concentrations, is critical in determining their theoretical oxygen transfer benefit.

Several methods, both theoretical and practical, have been utilized in the measurement of dissolved oxygen in pure PFC solutions and in PFC emulsions. Most of these methods require specialized laboratory equipment and techniques that are not easily performed and are prohibitive for acquiring accurate information about dissolved oxygen content in PFCs and PFC emulsions. For example, the early method of Wessler et al., where gas chromatography measurements were utilized to assess oxygen solubility in 25 pure PFCs, required numerous columns, a molecular sieve, pressure lock injectors, sealed gassing chambers, and high-temperature incubation environments (70−300 °C). Herein, we present two novel and complementary methods to accurately determine dissolved oxygen content within solutions, particularly PFC emulsions, by employing a stirred oxygen microchamber typically utilized for oxygen consumption measurements and an oxygen-consuming enzymatic reaction coupled to a modification of the Trinder reaction that provides a direct measure of oxygen concentration.

The Trinder reaction is a well-characterized method for the accurate determination of peroxide concentrations in solutions by coupling it with peroxidase and shuttling an electron to a reducible dye complex.[36] It has been implemented with different enzymatic reactions, as in this paper, for the determination of glucose concentration, as one example. In this particular method, it becomes a two-step oxidation reduction reaction that first converts glucose and oxygen into gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Subsequently, in the presence of peroxidase, a mixture of colorimetric dyes (3,5-dichloro-2-hydroxybenzoic acid (DCHBA) and 4-aminoantipyrine (4AAP) is reduced to form a visible color with a measurable optical density at 510 nm. The optical density is directly proportional to peroxide concentration and, thereby, to the oxygen and glucose concentrations present in the first step of the reaction.

In this work, we assessed dissolved oxygen concentration for solutions containing variable concentrations of emulsified PFC. Within sealed, stirred oxygen microchambers, the total dissolved oxygen concentration was assessed using the Trinder reaction. In addition, oxygen trace measurements were recorded during the course of the experiment, which were obtained using oxygen sensors placed within the selected chambers. The total oxygen concentration measured in the PFC solutions was compared to specific parameters of the measured oxygen depletion traces during the time course of the enzymatic reaction, specifically the time to reach complete oxygen depletion (T_{O_2}), the area under the oxygen depletion trace (AUC), and the linear portion of the oxygen depletion trace (m_{O_2}), representative of the zero-order reaction rate of the enzymatic complex. These oxygen trace parameters were then compared to the total oxygen concentration measurements, as well as previously published literature values,[1,11,15,37−39] to determine the best metric for determining dissolved oxygen concentration.

MATERIALS AND METHODS

Emulsion Manufacture. For comparison with literature values and its extensive characterization, perfluorodecalin (Fluoromed LLC) was selected for testing within emulsions. Pluronic polaxamer copolymers (F-68, F-127 BASF corp) were dissolved at 2% w/v in Hank's Balanced Salt Solution, (HBSS; Mediatech/Cellgro). This solution was continuously circulated through a model M 110-Y high-pressure pneumatic microfluidizer processor (Microfluidics) at 5000 PSI. The entire solution was cooled with an ice−water slurry to 4−8 °C. In a stepwise fashion, a volume of a pure perfluorodecalin was added to obtain the desired emulsion concentration (typically 10% v/v). The mixture was emulsified into a uniform micellar suspension for 8 min and then collected in a 250 cm^3 conical tube. The solution was adjusted to pH 7.35 and filtered through a sterile 0.2 μm filter.

Emulsion Characterization: PFC Quantification by Gravimetric Determination. Emulsion PFC content was quantified by gravimetric determination, performed by weighing 1 mL of each PFC emulsion and comparing the observed weight to a standard curve generated from serial 1 mL mixtures of the pure PFC and base solution at 0, 5, 10, 20, 40 and 100% v/v.

Solubility Determination: Trinder Reaction in Sealed Oxygen Microchamber. Stock solutions (20X) containing 10.5 U/mL glucose oxidase (Sigma Aldrich) and 5 U/mL peroxidase (Sigma Aldrich) were prepared in dPBS w/o Ca^{2+}/Mg^{2+} (Mediatech/Cellgro). Trinder reagent dyes, 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine were dissolved in HBSS at concentrations of 80 and 20 mg/mL, respectively (20X stock).

Standard curves of peroxide were prepared using serial dilutions in dPBS and a 1 mM peroxide stock. The peroxide concentration was determined spectrophotometrically using an extinction coefficient of 43.6 M^{-1} cm^{-1}.[40] For each standard concetration, 1 part of each dye was added per 18 parts peroxide standard into a 1.5 mL Eppendorf tube. The tubes were sealed and suspended in a 37 °C water bath. The reaction was allowed to continue for 10 min then stopped by addition of 10 μL of 1 M NaOH per 200 μL of solution. The tubes were vigorously mixed on a vortex for 10 s and then 100 μL samples were read in 96 well plates at 450 nm using 620 nm as the correction wavelength. dPBS without peroxide was utilized as the reaction blank,.

Solutions for solubility determination were prepared by adding 1 part of each dye per 18 parts of either control (HBSS + w% surfactant base solutions) or PFC emulsion. For determination of oxygen content, custom designed, stirred, thermoregulated oxygen-sensing titanium microchambers (6.4 mm i.d., ∼200 μL sealed chamber liquid volume, Instech Laboratories, Plymouth Meeting, PA) were utilized. The custom systems were designed with a ruthenium sol−gel based lifetime fluorescence spot sensor (PreSens GMBH, Regensburg, Germany) housed in the chamber

wall. Measurements were performed at either 37 or 25 °C, controlled via a circulating water bath (Haake). For each measurement, 300 μL of solution was added to the chamber. The chamber was capped with an acrylic plug containing a narrow injection port at the top. After capping, the final internal chamber volume was approximately 200 μL. The solution was allowed to equilibrate with the internal chamber temperature and the oxygen partial pressure was monitored/recorded every two seconds to ensure signal stability. At equilibration, 10 μL of the glucose oxidase/peroxidase enzyme solution was added to the chamber and the injection port immediately sealed with a custom acrylic plug. The chamber oxygen partial pressure was recorded every 2 s to monitor the reaction and for an additional 10 min after reaching zero to ensure reaction completion. The sample was then collected into a tube containing 10 μL of 1 M NaOH, vigorously mixed, and centrifuged at 20 000 rpm for 30 s in a microcentrifuge, to phase separate the hydrophobic emulsion components from the liquid volume. Controls were also centrifuged to maintain consistent sample handling. Absorbance of the reaction solutions was measured at 450 and 620 nm and the transfer ($abs_{450} - abs_{620}$) recorded. Dissolved oxygen content was determined by comparing against the peroxide standard curve, which is termed Trind[O_2] for clarity. Additionally, the fold increase/decrease in dissolved oxygen relative to the base solution control was determined by taking the ratios of measured oxygen concentrations.

The oxygen traces recorded from the sensing unit were graphed and analyzed. Parameters of particular interest were as follows: the time to reach zero oxygen (T_{O_2}), the area under the curve (AUC), and the slope of the zero-order reaction rate (linear portion of the oxygen depletion) from 140 to 40 mmHg (m_{O_2}) Control solution and emulsion data were compared relative to the collected concentration data. The fold (PFC/control) of these results was compared to the fold (PFC/control) of the measured oxygen concentration by means of the Trinder reaction.

Solubility Determination: Characteristic PFC Curves. For measurements performed at 37 °C, perfluorodecalin emulsions of 10%, 5%, and 2.5% v/v were prepared ($n = 3$) and the PFC concentration quantified by gravimetric determination as outlined above. For measurements made at 25 °C, a single v/v % emulsion was manufactured and perfluorodecalin content was determined by gravimetry. Serial dilutions of this emulsion were made at 1:2 and 1:4 with emulsion base solution. Solubility measurements were made for all emulsion solutions and the blank to generate characteristic dissolved oxygen curves at 37 and 25 °C. From extrapolation of the equation of these curves, the theoretical fold oxygen solubilities of the pure PFCs were determined by addition of the slope and the intercept. This results was subsequently compared to theoretical values derived via eq 1, as detailed by A. L. Rosen et al.[35]

$$[O_2]_{emulsion} = \{((\alpha_{PFC} \times \%_{PFC}) + (\alpha_{H_2O} \times (1 - \%_{PFC}))) \times pO_2\} \quad (1)$$

In this equation, α represents the Bunsen solubility coefficient for the various solution components, p_{O_2} is the oxygen partial pressure, % is the v/v percent of PFC in solution, and $[O_2]_{emulsion}$ is the concentration of dissolved oxygen in the emulsion solution.

RESULTS AND DISCUSSION

Emulsion Characterization: PFC Quantification by Gravimetric Determination. Gravimetric determination of perfluorodecalin content in each emulsion was performed to ensure the accuracy of solubility measurements and calculations when comparing against published literature values. Additionally, these values were utilized to determine loss, if any, during the emulsification process. The emulsions had a measured perfluorodecalin content lower than the expected values (9.05% ± 0.035% and 6.81% ± 0.042% at 37 and 25 °C, respectively, for 10% theoretical concentration). This is likely due to loss during initial priming and residual left in the system after collection. Figure 1 displays the standard curves utilized for v/v % perfluorodecalin determination, from the measured 1 mL weight of manufactured mixtures and from the theoretical values based on the published density of the pure PFC, 1.93 (Fluoromed, L.P., product specifications http://fluoromed.com/ProductsMain.htm), utilizing eq 2.

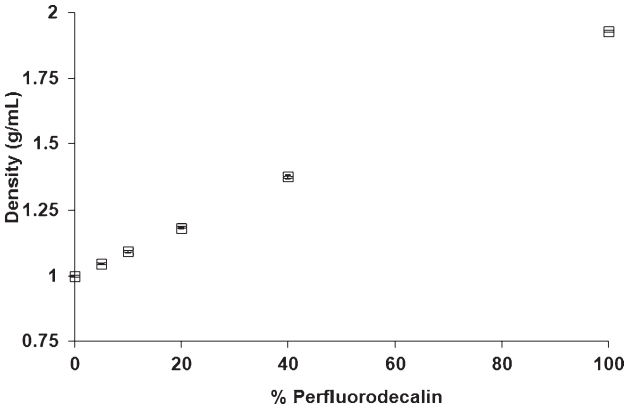


Figure 1. Gravimetric calibration curve for perfluorodecalin. Closed diamonds represent theoretical density values based on formula; open squares represent measured values.

$$\rho_{mixture} = \%_{base} \times \rho_{base} + \%_{PFC} \times \rho_{PFC} \quad (2)$$

where $\rho_{mixture}$ is the density of the PFC/base solution, ρ_{base} is the density of the base solution, and $\rho_{mixture}$ is the density of the PFC.

In comparing the measured standard curve to the theoretical curve, the two lines were statistically identical ($p = 0.95$) with the largest deviation, 0.004 g/mL, occurring in the 20% PFC mixture. The lines had equivalent goodness of fit, $R^2 = 1$, and was described by the following equation: $y = 0.0093x + 0.9991$. This result establishes the validity of the gravimetric method in accurately determining the emulsion PFC content.

Solubility Determination: Trinder Reaction Oxygen Concentration. In the presence of glucose oxidase, β-D-glucose reacts with oxygen and water to form D-gluconic acid and hydrogen peroxide. This peroxide formation can be readily quantified in the presence of peroxidase and electron-accepting colorimetric dye complexes. This reaction complex is detailed in eq 3.

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{D-glucose oxidase}} \text{gluconicacid} + H_2O_2$$
$$2H_2O_2 + 4AAP + 3,5\text{-DCHBA} \xrightarrow{\text{peroxidase}} \text{dyecomplex} + H_2O \quad (3)$$

To calculate the accuracy of our enzymatic method and to accurately quantify the dissolved oxygen concentration, serial dilutions of hydrogen peroxide were mixed with the dye complex, loaded into the sealed oxygen microchambers, and injected with the enzyme complex. The reaction was carried to completion, as detailed in the methods, and the resulting absorbance was

Table 1. Reported Fold Increase in Oxygen Solubilities in Perfluorodecalin and Perfluorodecalin Emulsions, Expressed as Fold Increase over PFC-Free Control Solutions at the Same Temperature

source	solution	temp (°C)	FOLD control (extrapolated range)
Reiss[8,19]	pure PFD	25	13.29−16.57
Kaisers[22]	pure PFD	25	16.28−20.3
Dias[26]	pure PFD	25	13.62−16.98
Wessler, Clark[1]	pure PFD/PFD	25	13.39−16.68
Lowe[15,18]	pure PFD	37	16.53−20.6

quantified. Standard curves (n = 5) relating hydrogen peroxide concentration to absorbance were consistently linear and the interassay standard curves were nearly identical with less than a 4% coefficient of variation in all standard concentrations. Standards ranged in concentration value from 62.5 to 750 μM with an approximate OD of 1.6 for the highest standards, indicating that higher concentrations could be utilized, if needed. Measured peroxide concentrations were then converted to be expressed as the concentration of oxygen in each measured solution volume, per the reaction kinetics outlined in eq 3. For clarity, this term is defined as $[O_2]_{Trind}$.

To evaluate the capacity of this method to quantify oxygen solubility, 3% v/v perfluorodecalin emulsion solutions, in addition to a PFC-free buffer solution, were tested at both 37 and 25 °C. Following termination of the experiment, absorbance readings were then converted to oxygen concentration, termed Trind[O_2], using the peroxide standard curve. To permit comparison, Trind[O_2] was normalized by the PFC-free control solution

To compare the accuracy of these measurements to published data, the theoretical fold concentrations were derived, using the values published in prior works, as detailed in Table 1. To determine these values, the solubility of the emulsion, SO_2(emulsion), was first calculated utilizing eq 4.

$$SO_2(\text{emulsion}) = \%\text{PFC} \times SO_2(\text{PFC}) + \%\text{base} \times SO_2(\text{base}) \quad (4)$$

where SO_2(PFC) and SO_2(base) are the theoretical solubility measurements for the PFC and PFC-free base solution, respectively.

Next, the theoretical solubility of the emulsion, SO_2(emulsion), was normalized by the theoretical solubility of the PFC-free control solution (HBSS) to permit comparison to experimental data. These values are summarized in the far right column of Table 1.

Solubility Determination: Trinder Reaction Oxygen Trace. In addition to terminal measurements of absorbance, oxygen traces were collected from the sealed chamber throughout the course of the enzymatic reaction. Oxygen partial pressure was detected by the lifetime fluorescence sensors built within the chamber walls and the measured values were recorded by the integrated software every 2 s. Figure 2 shows representative oxygen traces from control solutions (HBSS + dissolved surfactant), $^1/_2$ perfluorodecalin emulsion, and full perfluorodecalin emulsion measured following the addition of glucose oxidase/peroxidase enzyme solution. Consistently, the oxygen traces for each PFC dilution were reproducible and significantly different from the other PFC concentrations tested, with the same trend observed at both 25 and 37 °C. For the oxygen traces, three metrics were analyzed: T_{O_2}, time to zero oxygen; AUC, the integral of the oxygen trace (initial read to zero oxygen); slope, m_{O_2}, the oxygen trace from thermal equilibrium (140 mmHg) to the apparent Michaelis−Menten constant, and K_m, of the oxygen-depleting enzyme reaction.

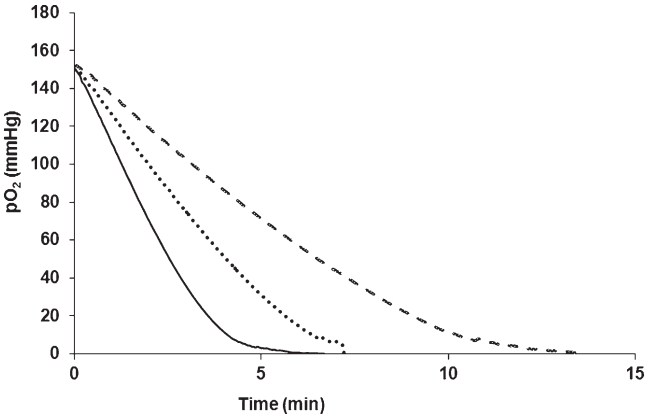


Figure 2. Average oxygen traces of, from left to right, base solution (solid line), $^1/_2$ perfluorodecalin emulsion (dotted line), pure perfluorodecalin emulsion (dashed line).

Following data collection, each parameter was normalized to the PFC-free control solution and compared to both the fold increase measured by the Trinder reaction, as well as the theoretical values.

Comparison of these variables associated with oxygen traces (T_{O_2}, AUC, and m_{O_2}) to the measured and theoretical fold concentrations found that the best metric of fold-oxygen solubility was the parameter m_{O_2}.

Solubility Determination: Characteristic PFC Curve. The published oxygen solubility values for perfluorodecalin range between 13- and 20-fold higher than water and physiological salt solutions (see Table 1), depending on the temperature of the measurements and the oxygen partial pressure (e.g., room air versus pure oxygen saturation). Figure 3, a and b, shows the measured oxygen solubilities relative to control solutions (HBSS) for perfluorodecalin emulsions at 37 and 25 °C. The measured fold m_{O_2} and $[O_2]_{Trind}$ are shown. Linear fits of the experimental data at 37 °C were 18.3x + 0.94 for m_{O_2} and 16.67x + 0.96 for Trind[O_2]. At 25 °C, linear fits of the experimental data were 12.491x + 1.042 for m_{O_2} and 13.369 + 1.036 for Trind[O_2]. R^2 ranged from a minimum of 0.981 for the m_{O_2} at 25 °C to a maximum of 0.997 for the m_{O_2} at 37 °C. Using the linear regression of both the slope and measured concentration curves, the dissolved oxygen capacity of pure perfluorodecalin was extrapolated as 17.59 and 14.41 for the measured concentration, Trind[O_2], and 19.28 and 13.53 for the measured slope, m_{O_2}, at 37 and 25 °C, respectively. As shown in Table 1, for the PFC type tested, perfluorodecalin, the measured increases in m_{O_2} and Trind[O_2] were well within the literature values referenced. These results indicate that both parameters are robust for determining dissolved oxygen content in PFC emulsions and other aqueous solutions.

As expected, the measured values of m_{O_2} and Trind[O_2] were found to be in strong agreement, as illustrated in Figure 4, where a strong correlation between the two values is evident. The value measured exhibited less than a 6% difference between measured fold concentration and measured fold linear slope. The linear fit

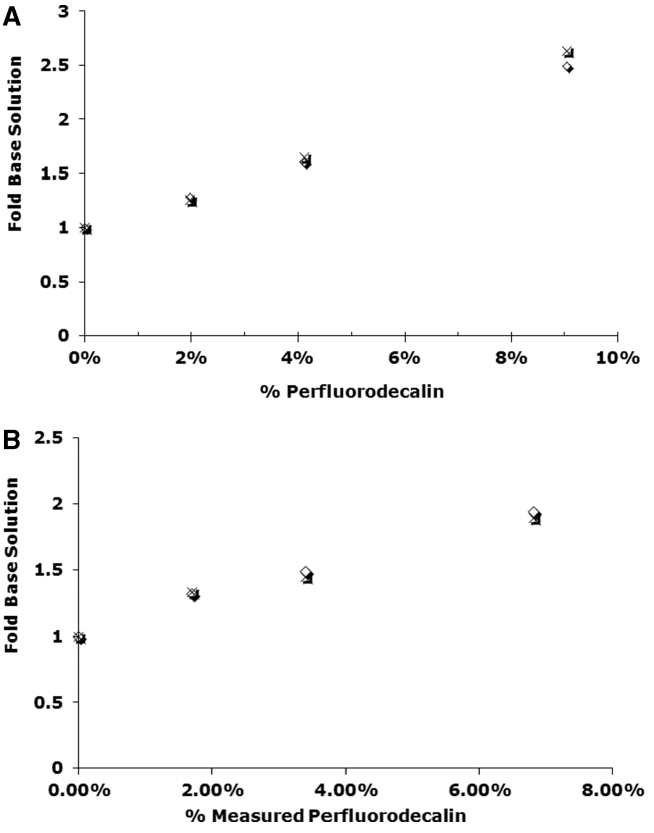


Figure 3. (A) Plot of measured fold concentration, Trind[O_2] (open diamonds), by Trinder reaction and fold measured slope, m_{O_2} (cross hairs), from oxygen traces at 37 °C. (B) Plot of measured fold concentration, Trind[O_2] (open diamonds) by Trinder reaction and measured fold slope, m_{O_2} (cross hairs), from oxygen traces at 25 °C.

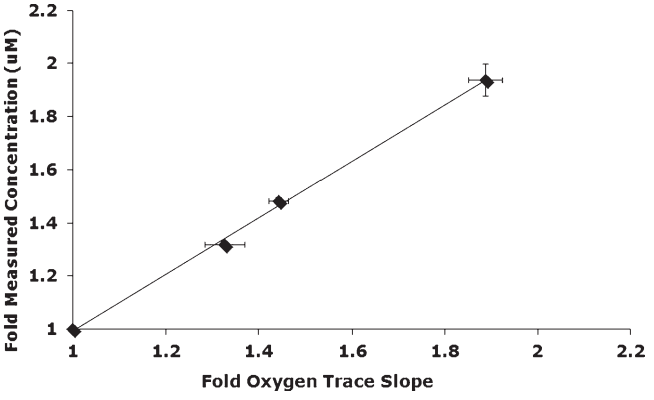


Figure 4. Measured fold slope, m_{O_2}, vs measured fold concentration, Trind[O_2], of perfluorodecalin emulsions relative to base solution.

was described by the equation, $y = 1.06x - 0.08$ with an R^2 value of 0.998. This further verifies that the two metrics can be confidently interchanged in determining the dissolved oxygen content in perfluorodecalin emulsions.

CONCLUSIONS

PFC emulsions are a promising tool for improving oxygen transfer within solutions and biomaterials; however, thorough characterization of their potential oxygen mass transfer enhancements is limited. Critical to the determination of the expected benefit of these emulsions is the measurement of the dissolved oxygen content in the emulsions and, less so, the effective oxygen diffusivity through the emulsions. The product of these two characteristics is the diffusive permeability coefficient, which is the direct assessment of oxygen mass transfer through any system. Determination of the diffusive permeability allows for numerical optimization of emulsion preparations to maximize both characteristics of oxygen diffusive permeability and thereby, oxygen transfer.

Prior methods for the accurate determination of dissolved oxygen content within solutions often required sophisticated laboratory equipment with custom glassware and precision manometers or gas chromatographs. Additionally, many methods are quite laborious and time consuming, requiring hours to collect a single measurement.[37,40] Herein, we present a novel method with complementary results that, while requiring a piece of custom equipment, is more user friendly and efficient. Our data also illustrate that the use of recorded oxygen traces within these sealed system is as accurate as colorimetric determination via glucose oxidase dependent oxygen consumption coupled to the Tinder reaction, thereby allowing for further ease in measurements by oxygen traces alone. Our results demonstrate that the oxygen solubility measurements utilizing our system fall within published values collected using more intricate methodologies, thereby offering a simpler means of quantifying dissolved oxygen in any solution or micellar suspension, with some modifications. Utilization of this method permits accurate determination of oxygen content in solutions used for medical applications and oxygen delivery capacities. Importantly, the methods utilized in this study are technically more appealing than conventional methods for solubility measurements. Other groups have used technically demanding permutations of this reaction for oxygen determination in nonaqueous solutions and emulsions in the past, but measuring the consumption of glucose as opposed to oxygen.[41,42] Furthermore, this method could be translated to the measurement of other gases, such as carbon dioxide, which, given the benefit of PFC in enhancing the transport of CO_2, would be a useful measurement for characterizing PFC emulsions.[43] Enzymatic reactions, such as phosphoenolpyruvate carboxylase in conjunction with fast Violet B, could be used to permit absorbance readings, with the fitting of sealed chambers with CO_2 sensors for temporal measurements also feasible.[44]

This simple method would benefit researchers in the optimization of the manufacture of oxygen-carrying solutions utilized in, for example, wound healing, blood replacement therapies, and the immuno-isolation of cell products for transplant therapies in the treatment of medical conditions such as diabetes mellitus. We feel that this simple assay could serve a critical role in the advancement of methods for enhanced oxygen delivery.

AUTHOR INFORMATION

Corresponding Author

*Phone: (305) 243-9768. Fax: (305) 243-4404. E-mail: cstabler@med.miami.edu.

ACKNOWLEDGMENT

This work was supported by the Diabetes Research Institute Foundation (www.diabetesresearch.org) and the Children with Diabetes Foundation (www.cwdfoundation.org). We thank

Michael and Paul Loughnane of Instech Laboratories for their custom-designed oxygen chambers and for providing CAD schematics.

REFERENCES

(1) Clark, L. C., Jr.; Wesseler, E. P.; Kaplan, S.; Miller, M. L.; Becker, C.; Emory, C.; Stanley, L.; Becattini, F.; Obrock, V. *Fed. Proc.* **1975**, *34*, 1468.
(2) Hall, C. A. *Fed. Proc.* **1975**, *34*, 1513.
(3) Rosenblum, W. I. *Fed. Proc.* **1975**, *34*, 1493.
(4) Kostrzewska, E. *Ann. R. Coll. Surg. Engl.* **1976**, *58*, 115.
(5) Novakova, V. *Acta Physiol. Scand.* **1976**, *98*, 433.
(6) Doss, L. L.; Kaufman, N.; Bicher, H. I. *Microvasc. Res.* **1977**, *13*, 253.
(7) Clark, L. C., Jr. *Prog. Clin. Biol. Res.* **1978**, *19*, 69.
(8) Riess, J. G.; Le Blanc, M. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 621.
(9) Bowman, R. J. *Hum. Pathol.* **1983**, *14*, 218.
(10) Riess, J. G. *Life Support Syst.* **1984**, *2*, 273.
(11) Clark, L. C., Jr. *Int. Anesthesiol. Clin.* **1985**, *23*, 1.
(12) Gould, S. A.; Sehgal, L. R.; Rosen, A. L.; Sehgal, H. L.; Moss, G. S. *Ann. Emerg. Med.* **1985**, *14*, 798.
(13) Waxman, K. *Ann. Emerg. Med.* **1986**, *15*, 1423.
(14) Biro, G. P.; Blais, P. *Crit. Rev. Oncol. Hematol.* **1987**, *6*, 311.
(15) Lowe, K. C. *Comput. Biochem. Physiol. A Comput. Physiol* **1987**, *87*, 825.
(16) Faithfull, N. S. *Adv. Exp. Med. Biol.* **1992**, *317*, 441.
(17) Spahn, D. R. *Crit. Care* **1999**, *3*, R93.
(18) Lowe, K. C. *Expert Opin. Pharmacother.* **2001**, *2*, 1057.
(19) Riess, J. G. *Artif. Cells Blood Substit. Immobil. Biotechnol.* **2006**, *34*, 567.
(20) Biro, G. P. *Transfus. Med. Rev.* **1993**, *7*, 84.
(21) Faithfull, N. *Adv. Exp. Med. Biol.* **1992**, *317*, 55.
(22) Kaisers, U.; Kelly, K. P.; Busch, T. *Br. J. Anaesth.* **2003**, *91*, 143.
(23) Navari, R. M.; Rosenblum, W. I.; Kontos, H. A.; Patterson, J. L., Jr. *Res. Exp. Med. (Berlin)* **1977**, *170*, 169.
(24) Patel, S.; Mehra, A. *ASAIO J.* **1998**, *44*, 144.
(25) Shah, N.; Mehra, A. *ASAIO J.* **1996**, *42*, 181.
(26) Dias, A. M. A.; Freire, M.; Coutinho, J. A. P.; Marrucho, I. M. *Fluid Phase Equilib.* **2004**, *222*−*223*, 325.
(27) O'Brien, R. N.; Langlais, A. J.; Seufert, W. D. *Science* **1982**, *217*, 153.
(28) Urushihara, T.; Sumimoto, K.; Ikeda, M.; Yamanaka, K.; Hong, H. Q.; Ito, H.; Fukuda, Y.; Dohi, K. *Biomater. Artif. Cells Immobilization Biotechnol.* **1992**, *20*, 933.
(29) Holman, W. L.; McGiffin, D. C.; Vicente, W. V.; Spruell, R. D.; Pacifico, A. D. *Artif. Cells Blood Substit. Immobil. Biotechnol.* **1994**, *22*, 979.
(30) Johnson, E. C.; Erickson, B. K.; Podolsky, A.; Birks, E. K.; Keipert, P. E.; Faithfull, N. S.; Wagner, P. D. *J. Appl. Physiol.* **1995**, *79*, 1777.
(31) Johnson, A. S.; O'Sullivan, E.; D'Aoust, L. N.; Omer, A.; Bonner-Weir, S.; Fisher, R. J.; Weir, G. C.; Colton, C. K. *Tissue Eng. Part C Methods* **2011**, *17*, 435.
(32) Chin, K.; Khattak, S.; Bhatia, S.; Roberts, S. *Biotechnol. Prog.* **2008**, *24*, 358.
(33) Bleta, R.; Blin, J. L.; Stebe, M. J. *J. Phys. Chem. B* **2006**, *110*, 23547.
(34) Freire, M. G.; Gomes, L.; Santos, L. M.; Marrucho, I. M.; Coutinho, J. A. *J. Phys. Chem. B* **2006**, *110*, 22923.
(35) Rosen, A. L.; Sehgal, L. R.; Gould, S. A.; Sehgal, H. L.; Moss, G. S. *Int. Anesthesiol. Clin.* **1985**, *23*, 95.
(36) Trinder, P. *J. Clin. Pathol.* **1969**, *22*, 158.
(37) Faithfull, N. S. *Artif. Cells Blood Substit. Immobil. Biotechnol.* **1994**, *22*, 181.
(38) Riess, J. G. *Artif. Cells Blood Substit. Immobil. Biotechnol.* **2005**, *33*, 47.
(39) Faithfull, N. *Biomater. Artif. Cells. Immobilization Biotechnol.* **1992**, *25*, 797.
(40) Noble, R. W.; Gibson, Q. H. *J. Biol. Chem.* **1970**, *245*, 2409.
(41) Freire, M. G.; Dias, A.; Coehlo, M. A. Z.; Coutinho, J. A. P.; Marrucho, I. M. *Fluid Phase Equilib.* **2005**, *231*, 109.
(42) Ghosh, A.; Janic, V.; Sloviter, H. A. *Anal. Biochem.* **1970**, *38*, 270.
(43) Lowe, K.; Davey, M.; Power, J. *Trends Biotechnol.* **1998**, *16*, 272.
(44) Bandurski, R. S.; Greiner, C. M. *J. Biol. Chem.* **1953**, *204*, 781.