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Enzymatic method for determining oxygen solubility in perfluorocarbon emulsions

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Abstract

An enzymatic method to measure the glucose content in solution was adapted for measurements of oxygen content in oil in water emulsions with a precision of about 1%. The oxygen solubility in concentrated perfluorocarbon in water emulsions at 310.2 K and atmospheric pressure was measured. To study the effect of the perfluorocarbon and of the surfactant in the oxygen solubility, two perfluorocarbons, *n*-perfluorohexane and perfluorodecalin, in combination with three nonionic surfactants, Lecithin, Span 20 and Pluronic F-68 were used. The concentrations used were 50% (w/v) for the perfluorocarbons and 5% (w/v) for the surfactant. The oxygen solubility is shown to be independent of the surfactant used and dependent only of the perfluorocarbon employed in the emulsions studied.

Keywords: Solubility measurements; Oxygen; Perfluorocarbon emulsions; Enzymatic method

1. Introduction

Perfluorocarbons (PFCs) are fluorinated, inert organic compounds that can dissolve large volumes of respiratory gases such as oxygen and carbon dioxide [1–6]. The oxygen solubility in liquid PFCs used for blood substitutes is large when compared, for example, to the same gas in water [4,6].

Since Clark and Gollan [7] demonstrated the capacity of liquid perfluorocarbons to support animal life by liquid breathing, intensive research on perfluorocarbons has been developed aiming especially at their use as oxygen carriers, in artificial blood substitutes. Other biomedical applications for perfluorochemicals and their emulsions include their use as pump-priming fluids for cardiopulmonary bypass, lung ventilation fluids, anti-cancer agents, lubrication and cushioning for articular disorders, organ perfusates and cell culture media supplements, diagnostic imaging agents, ophthalmologic tools and drug formulations and delivery [1–6]. Beside their importance in the biomedical field [8–10] they can also be used in a wide variety of areas such as surfactants in supercritical solvents, environmental probes, anticorrosive and antifriction components, flame retardants, water repellents or sliding agents, in paints, coatings, polymer technology, metal working and uranium recovery process [11–13].

Since PFCs are immiscible in aqueous systems, including biological fluids, they must be converted to an emulsified form to be safely injected into the blood vasculature. It is thus essential to determine the amount of oxygen that a PFC-in-water emulsion can dissolve and to develop a precise and expedite method to determine it.

To the best of our knowledge few works have been published on the description of physical or chemical methods, which are known to be the most accurate, to measure the solubility of gases in emulsions. Most papers dealing with these solubility phenomena fall in the biomedical field or in the biotechnological area and use commercial apparatuses for this purpose. In these areas the effect of PFCs is not accurately measured since the usual oxygen electrode used often fail to accurately account for the gas in the organic phase that contains substantially more oxygen than the aqueous phase in a heterogeneous system [14]. There are several studies in

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the field of oxygen transfer through water in organic emulsions and in the development of theoretical models on the volumetric oxygen uptake and volumetric mass transfer coefficient, but they do not present oxygen solubility data for these dispersions [15–19].

On the other hand, King and co-workers [20–25] have extensively studied the solubility of gases in micellar aggregates in aqueous solutions. The fact that no organic phase other than the surfactant was present, and thus the gas solubility was very low, lead King and co-workers to develop a new method to measure the gas solubility, involving a step where both the solution and the gas equilibrate at an elevated pressure (close to 20 bar). Analyzing the solubility data obtained, they found that the micellar gas solubility depends strongly on the conditions inside the micelle such as the size and nature of the surfactant tail group and is only mildly affected by the conditions outside the micelle, e.g. salinity and/or the nature of the surfactant headgroup.

Since the oxygen content of samples of dispersed perfluorochemicals cannot be measured by conventional methods, Ghosh et al. [26] proposed an enzymatic method for measuring oxygen in such nonaqueous materials. This method is based in the oxidation of glucose by molecular oxygen catalyzed by glucose oxidase that is commonly used for dosing glucose when oxygen in excess is present [27]. In this work, a method based on a similar approach is used to accurately measure the oxygen content in several perfluorocarbon emulsions. This new method uses a different combination of enzymes, which has the advantage of simplifying the experimental procedure, while increasing its precision.

2. Materials and methods

The perfluorocarbons used were *n*-perfluorohexane (C_6F_{14}) and perfluorodecalin ($C_{10}F_{18}$), both 95% pure and acquired from Flutec (PP6 and PP1, respectively). Three nonionic surfactants were tested: lecithin (L-α-phosphatidylcholine) from egg yolk with an average purity of 88.6% (acid value < 25, iodine value < 80, peroxide value < 5) from Fluka, Pluronic F-68, 10% aqueous solution, and Span 20 both from Sigma–Aldrich. Fluorocarbons and emulsifiers were used without further purification. Deionized and double distilled water was used in the preparation of the emulsions.

Emulsions of 50% (w/v) of each perfluorocarbon in water using 5% (w/v) of one of the three different surfactants were prepared by sonication, using an *IKA Labortechnik* sonicator, model *U200S* control. The sonication was performed for 2 min, at cycle 1, with a constant amplitude of 80%, keeping the tube immerse in ice to avoid heating of the emulsions, made up to a final volume of 10.0 ml each. The composition of the studied emulsions is described in Table 1.

The emulsions stability was studied through the evolution of the mean particle size with time. The droplets diameter was analyzed with an optic microscope, *Nikon*, model *Eclipse 200*, with a digital camera, *Nikon Coolpix 990*. These images were processed and analyzed with a program developed in *Matlab*® *6.1* for this purpose [28]. The emulsions were prepared and placed in a thermostatic oven (±0.5 K) for 42 days. For each emulsion, the droplet diameter was measured periodically for 42 days. At each time an average of 1267 and 4102 particles for the *n*-perfluorohexane and perfluorodecalin emulsions, respectively, was analyzed. The larger number of globules analyzed in perfluorodecalin emulsions is due to their smaller size. Since the precision of the program decreases with the decreasing size of objects a larger number was analyzed to reduce the systematic errors. A statistical analysis of the experimental data aiming at assuring that no systematic errors (or bias) were done during the particles diameter measurements was performed. When no systematic errors are present, the population follows a Gaussian distribution.

Table 1
Composition of the emulsions studied

Emulsion	Perfluorocarbon (50%, w/v)	Surfactant (5%, w/v)
1	C_6F_{14}	Lecithin
2	C_6F_{14}	Span 20
3	C_6F_{14}	Pluronic F-68
4	$C_{10}F_{18}$	Lecithin
5	$C_{10}F_{18}$	Span 20
6	$C_{10}F_{18}$	Pluronic F-68

Emulsions were saturated with room air for 30 min and their oxygen content measured. The use of room air instead of oxygen is due to the fact that the first renders an easier experimental procedure. Also, comparison of the solubility of oxygen in pure PFCs saturated with air to pure PFCs saturated with pure oxygen do not exhibit significant differences [29]. The oxygen content in the studied emulsions was measured with an enzymatic method based on the oxidation of glucose by molecular oxygen catalyzed by glucose oxidase. This method is commonly used for dosing glucose when oxygen in excess is present [27] and was here adapted to measure the molecular oxygen when the glucose is the excess reactant. It can be described by the following equations:

$$\text{D-glucose} + H_2O + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-gluconic acid} + H_2O_2$$

$$H_2O_2 + \underset{\text{(colorless)}}{\text{reduced } o\text{-dianisidine}} \xrightarrow{\text{peroxidase}} \underset{\text{(brown)}}{\text{oxidized } o\text{-dianisidine}}$$

$$\underset{\text{(brown)}}{\text{oxidized } o\text{-dianisidine}} \xrightarrow{H_2SO_4} \underset{\text{(pink)}}{\text{oxidized } o\text{-dianisidine}}$$

The reagents for the application of the enzymatic method, Glucose (GO) Assay Kit, were acquired at Sigma–Aldrich. This kit contains glucose oxidase/peroxidase and *o*-dianisidine reagents and a glucose standard solution.

All the kit reagents were degassed before used, with a method consisting of successive melting/freezing cycles

while vacuum pumping noncondensable gases [30] to assure that the only source of oxygen was the emulsion. A 12 mol dm^{-3} solution of sulphuric acid from Riedel-de-Haën (95–97% pure) was prepared in deionized and double distilled water. The first two reactions took place in a thermostatic water bath at 310.2 (±0.5) K for 30 min. The reactions were stopped by the addition of the sulphuric acid solution. The intensity of the pink color of the supernatant solution against the blank, after 30 min of centrifugation at 3000 rpm, was measured at 540 nm with a *Shimadzu* spectrophotometer, model *UV-160A*. The concentration of the reduced oxygen present in each emulsion was calculated with a calibration curve. At least three measurements in three independent emulsions were made and standard deviations calculated.

3. Results and discussion

An example of the microscopic images obtained for the studied emulsions is presented in Fig. 1, for emulsion 4 at the initial and final state. The images obtained throughout the 42 days of experiment were analyzed with the developed program and the respective histograms were produced. The Gaussian behavior was observed in all the cases studied, as for example is depicted in Fig. 2 for emulsion 4. From this study it can be concluded that all the emulsions are stable within the 42 days period and that the droplet size does not significantly change with time. Since the amount of oxygen dissolved in perfluorocarbon emulsions is independent of the mean particle size of the emulsions, for the same perfluoro-

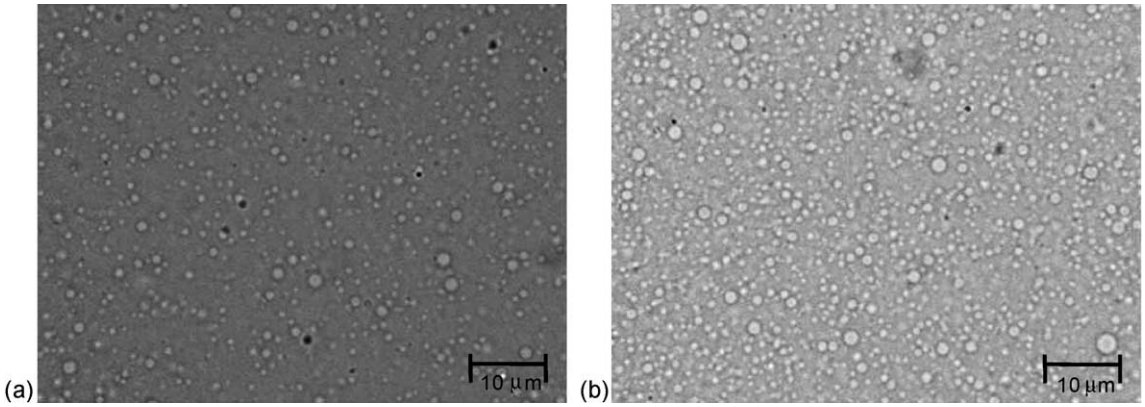

Fig. 1. Microscopic images for freshly prepared (a) and after 42 days (b) for emulsion 4 at 310.2 K.

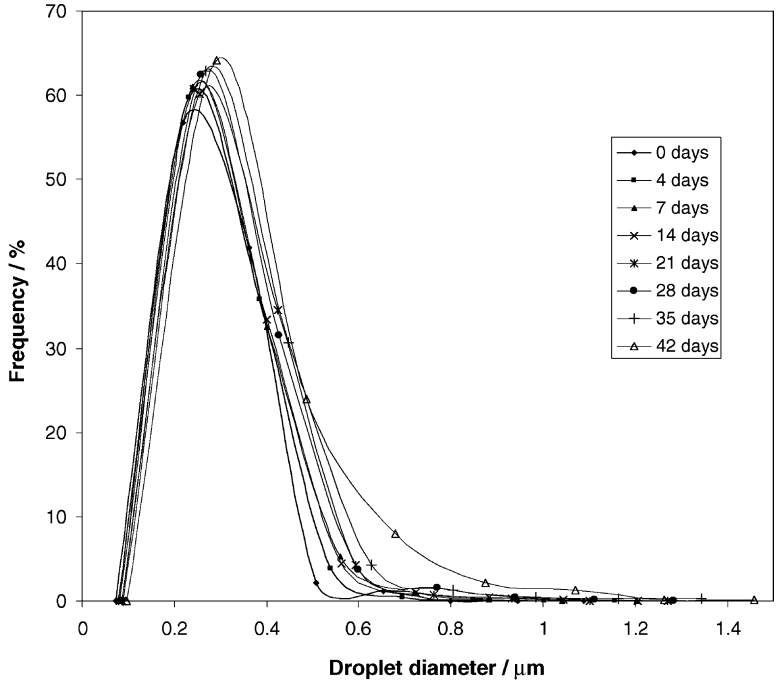

Fig. 2. Normal distributions function of the droplet size for emulsion 4 at 310.2 K, at several time periods.

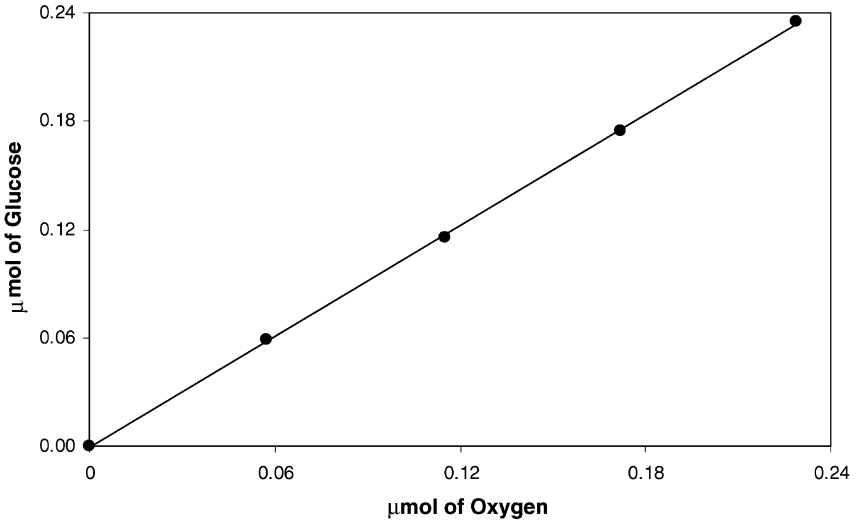

Fig. 3. Amount of glucose oxidized as function of amount of oxygen added. Exp. data, ●; solid line, linear regression.

carbon [31], the evaluation of the oxygen solubility was only performed in fresh emulsions.

Fig. 3 shows the linear relationship between the amount of glucose oxidized and the amount of oxygen added, indicating the validity of the enzymatic method to measure the dissolved oxygen in perfluorocarbon emulsions. The oxygen source was water equilibrated with air at 310.2 K for 30 min, which was added in varying amounts. The slope of the linear plot indicates that the molar ratio of glucose to oxygen in this experiment was 1.0 with a correlation factor of 0.999. This test was performed in order to verify if catalase was present in the commercial kit used. This enzyme leads to the decomposition of H_2O_2 formed in the glucose oxidase reaction yielding more molecular oxygen and thus giving molar ratios of glucose to oxygen greater than 1.0 [26].

The calibration curve used to calculate the amount of glucose that has reacted is presented in Fig. 4. The absorbance of the samples was measured at 540 nm. The concentration of oxygen present in each emulsion saturated with atmospheric air can be determined from Fig. 4, taking into account that the molar ratio of glucose/oxygen is 1.0. The concentration of oxygen in the n-perfluorohexane and perfluorodecalin in water emulsions using the enzymatic method at 310.2 K is presented in Table 2 . Since the experimental error associated to this method is in the order of 1%, the results reported show that although the oxygen solubility is independent of the surfactant, it depends on the PFC used in the emulsions. The perfluorodecalin emulsions dissolve more oxygen than the perfluorohexane ones, in terms of mole fraction, following the same trend observed for the pure PFCs. Sharts and Reese [32] observed the same fact for other perfluorocarbon emulsions.

Using experimental data from literature for the solubility of oxygen in pure liquid perfluorocarbons [4] and in water [6],

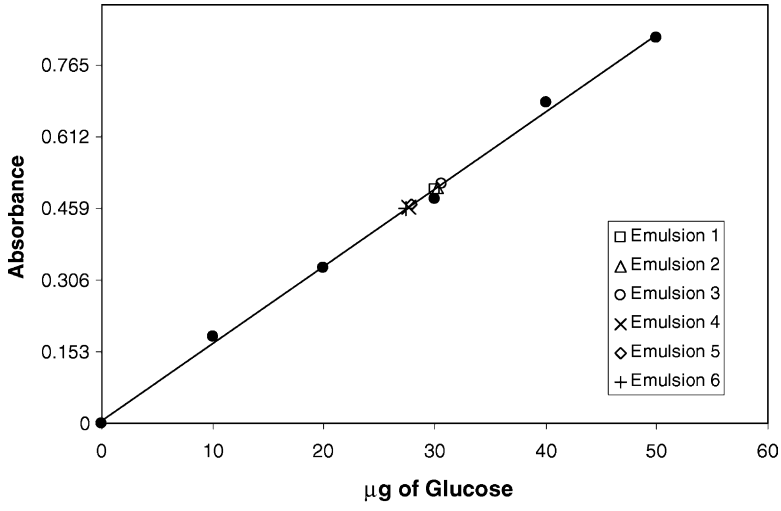

Fig. 4. Standard plot for absorbance at 540 nm as function of the glucose present (Exp. data, ●; solid line, linear regression) and samples interpolation.

Table 2
Moles of oxygen per ml of PFC emulsion, saturated with atmospheric air and the respective expected value at 310.2 K

Emulsion	Dissolved oxygen $\pm\sigma$ (μmol)[a]	Expected dissolved oxygen (μmol)[b]	Difference $\pm\sigma$ (%)[c]
1	0.99 ± 0.01	1.04	4.57 ± 0.79
2	1.00 ± 0.02	1.04	4.2 ± 2.8
3	1.00 ± 0.02	1.04	3.5 ± 3.0
4	0.94 ± 0.03	1.02	7.9 ± 3.7
5	0.95 ± 0.01	1.02	$6.9_0 \pm 0.82$
6	0.93 ± 0.02	1.02	8.5 ± 2.8

[a] Experimental dissolved oxygen and associated standard deviation using the enzymatic method.
[b] O_2 dissolved in the pure PFC [4] + O_2 dissolved in the pure water [6].
[c] Difference between the experimental and expected values.

the oxygen solubility in 50% (w/v) *n*-perfluorohexane and perfluorodecalin in water emulsions at the studied temperature of analysis was estimated. The values are also shown in Table 2. These values are slightly larger than the corresponding oxygen values found with the enzymatic method. Similar results were obtained by Palavra and co-workers [33], who compared the solubility of argon in water, pure dodecane and in aqueous solutions of sodium dodecyl sulfate (SDS). Argon's solubility in the SDS solution was 2.5 times lower then in pure dodecane but, two orders of magnitude higher than in pure water. According to the authors the results suggested that water penetration in the hydrocarbon chains of the micelle was responsible by the difference in solubility observed in the presence of micelles and in dodecane.

4. Conclusions

A suitable and expedite enzymatic method was adapted for measuring the amount of oxygen dissolved in perfluorocarbon-in-water emulsions at 310.2 K and atmospheric pressure.

The oxygen solubility is fairly independent of the surfactant used and dependent of the perfluorocarbon used in the studied emulsions. The perfluorodecalin emulsions dissolve more oxygen than the perfluorohexane ones, following the same trend observed in the pure PFCs.

The decrease in oxygen solubility of about 6% in the studied emulsions compared to the pure liquids can be explained by the dissolution of water in the organic phase hampering the oxygen dissolution in the same phase.

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