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Determination of Oxygen Solubility in Perfluorocarbon Emulsions by a Modified Enzymatic Method

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Abstract: Oxygen solubility in perfluorocarbon (PFC) emulsions is a crucial performance metric in fields such as artificial blood, oxygen transportation and diagnostic imaging agents. However, there is a lack of simple and accurate detection methods. In this study, the perfluorooctyl bromide (PFOB) emulsion was prepared by using perfluorohexylethyl polyoxyethylene ether (FEO) as the emulsifier, and a modified enzymatic method was developed by using a more stable Orange G as the color indicator to determine the oxygen solubility in the PFOB emulsion at 298.15 K and atmospheric pressure. For 0.2 mL emulsion, the oxygen solubility measured by the modified enzymatic method was close to that measured by gas-liquid chromatography as reported in the literature. The effects of mass fractions of PFC and FEO in the emulsions on the oxygen solubility were studied based on the modified enzymatic method. The results indicate that the oxygen solubility in the emulsion increases with the increasing mass fraction of PFC, independent of the mass fraction of the emulsifier.

Keywords: perfluorocarbon (PFC) emulsion; oxygen solubility; enzymatic method
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0 Introduction

Perfluorocarbons (PFCs) are fluorine-containing inert compounds that can dissolve significant amounts of oxygen and carbon dioxide owing to weak van der Waals interactions between PFC molecules[1-6]. They are often formulated into emulsions for applications in drug transportation, artificial blood, diagnostic imaging agents and so on[4-8]. The oxygen solubility in PFC emulsions is a crucial performance metric. However, it is difficult to measure precisely.

Methods for testing the dissolved oxygen in water include the iodometry, oxygen electrode method, and fluorescence quenching method[9]. The iodometry is a chemical titration that suffers from low accuracy and sensitivity, as well as high susceptibility to external interference[10-11]. The oxygen electrode method is not suitable for heterogeneous systems where non-aqueous solvents dissolve more oxygen than aqueous phases[12]. The fluorescence quenching method imposes high instrumental requirements, and the experimental data can be affected by the color of the emulsion[13]. Due to the interference among phases in the heterogeneous emulsion, the above methods cannot quickly and accurately measure the oxygen solubility in the PFC emulsion.

Ghosh et al.[12] developed an enzymatic method to measure the oxygen solubility in heterogeneous liquid phases. The enzymatic method is based on the glucose oxidase catalyzing the consumption of molecular oxygen by glucose to determine the dissolved oxygen in the emulsion. A colorimetric indicator derived from the pigments in the Glucostat reagent is used. The reaction is

$$\text{glucose} + O_2 + H_2O \longrightarrow \text{gluconolactone} + H_2O_2.$$

The enzymatic method is generally used to test glucose levels in oxygen-rich environments, which leads to relatively poor accuracy.

Freire et al.[14] improved the enzymatic method by using reduced *o*-dianisidine instead of the indicator dye in the finished reagent, and simplified the experimental steps for measurement. The reaction is

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

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

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

The improved enzymatic method utilizes glucose oxidase and peroxidase. Glucose oxidase consumes oxygen to produce hydrogen peroxide, and peroxidase catalyzes the reaction of hydrogen peroxide to consume reduced *o*-dianisidine[15]. Quantification of hydrogen peroxide via absorbance measurement enables the calculation of the

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amount of the consumed oxygen. However, reduced *o*-dianisidine is an unstable color indicator that may be oxidized by other substances during testing, complicating the experimental procedure and reducing the testing accuracy.

To address this issue, this paper develops a modified enzymatic method by using a more stable Orange G as the color indicator to determine the oxygen solubility in perfluorooctyl bromide (PFOB) emulsions with perfluorohexylethyl polyoxyethylene ether (FEO) as the emulsifier. Based on this method, the effects of mass fractions of PFC and FEO on the oxygen solubility in the emulsion are studied.

1 Materials and Methods

1.1 Materials

PFOB ($C_8F_{15}Br$) with a purity of 98% and glucose with a purity no less than 99.5% were purchased from Shanghai Adamas Reagent Co., Ltd. (China) and Shanghai Yuanye Bio-Technology Co., Ltd. (China), respectively. FEO with a purity of 95% and Orange G with a purity no less than 96% were supplied by Shanghai Aladdin Biochemical Technology Co., Ltd. (China). Glucose oxidase was provided by Shanghai Yuanye Bio-Technology Co., Ltd. (China) and ferrous sulfate was from Shanghai Aladdin Biochemical Technology Co., Ltd. (China).

1.2 Emulsion preparation

The emulsions were prepared by using a high-speed homogenizer (Fluke F10, Shanghai Youyi Instrument, China). A mixture of PFOB and FEO (as the emulsifier) was added gradually to water in the homogenizer within 30 s. The mixture was then processed at a speed of 5 000 r/min for 5 min.

To determine the oxygen solubility in the emulsion and study the effects of mass fractions of PFOB and FEO on the oxygen solubility, different emulsions were prepared as shown in Table 1.

Table 1 Composition of emulsions

Emulsion	PFOB mass fraction/%	FEO mass fraction/%
1	5.4	10
2	10.5	10
3	20.0	10
4	28.6	10
5	36.3	10
6	20.0	5
7	20.0	15

1.3 Emulsion particle size measurement

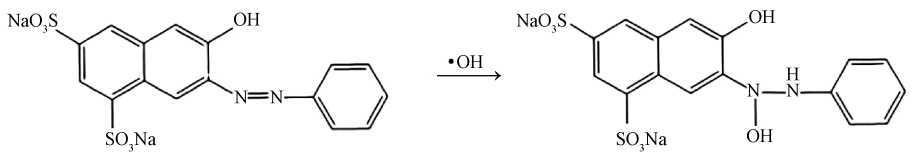
The aging mechanism of the emulsion was studied by examining changes in the emulsion particle size. Particle sizes were analyzed by using a 7XB-PC optical microscope from Shanghai Optical Instrument Factory, China. Emulsion particle sizes were continuously measured over a period of 10 d, and data were processed by using the Origin software for log-normal distribution analysis of the particle size distribution for each day.

1.4 Emulsion oxygen solubility measurement

As there is no difference between the oxygen solubility in emulsions saturated with air and that saturated with pure oxygen[16], the emulsions used for testing in this study were all saturated with air, thus simplifying the experimental procedure. The reactions are

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

$$Fe(\text{Ⅱ}) + H_2O_2 \longrightarrow Fe(\text{Ⅲ}) + {\cdot}OH + OH^-,$$



Orange G

Firstly, *D*-glucose is oxidized by oxygen to generate hydrogen peroxide. Then, the hydrogen peroxide reacts with Fe^{2+} through the Fenton reaction to form hydroxyl radicals. Finally, the hydroxyl radicals react with Orange G[17].

In the modified enzymatic method, 0.2 mL emulsion was mixed with 0.8 mL glucose oxidase solution and anaerobic water, and diluted to 1.0 mL. Then, the mixture was reacted with a solution containing Fe^{2+} and Orange G at a molar ratio of 1∶1 in a vacuum. The reaction was terminated after 2 h by heating to 80 ℃. After cooling, the absorbance at 478 nm was measured by using a DR6000 spectrophotometer (HACH, Germany), and the dissolved oxygen was calculated based on the standard curve of Orange G in Fig. 1.

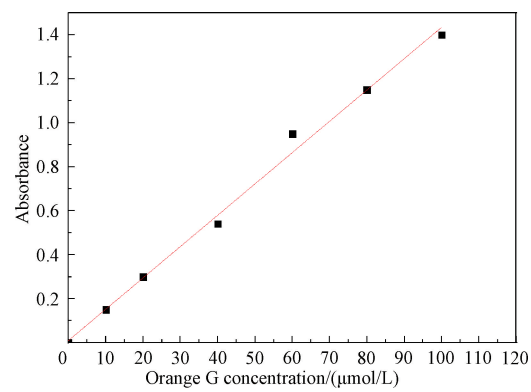


Fig. 1 Standard curve of Orange G

Due to the high costs and difficulties in storing the reagents used in the previous method, we conducted a literature comparison. The comparison between the previous enzymatic method and the modified enzymatic method is shown in Table 2.

Table 2 Comparison of different oxygen solubility detection methods

Method	Indicator sensitivity	Indicator stability	Cost	Experimental procedure	Reaction rate
Modified enzymatic method	High	Stable	Low	Simple	Fast(free radical reaction)
Previous enzymatic method	Middle	Unstable	High	Complex	Slow

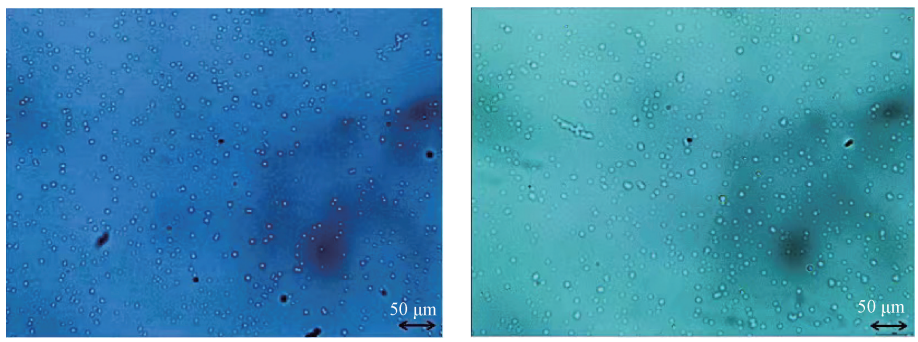
2 Results and Discussion

2.1 Properties of PFOB emulsion

The particle size of an emulsion refers to the average diameter of the dispersed phase in the continuous phase of the emulsion, and affects the stability and functionality of the emulsion. Analyzing the particle size distribution could provide more information for evaluating the quality and stability of the emulsion. Oxygen solubility is a key property of the emulsion and influences its application in artificial blood.

2.1.1 Particle size distribution of PFOB emulsion

Microscopic images of emulsion 3 from freshly prepared to the ninth day are shown in Fig. 2. The particle size increases on the ninth day. A log-normal distribution[18] obtained by measuring, statistically analyzing, and fitting the emulsion particle size data is shown in Fig. 3.



(a) (b)

Fig. 2 Microscopic images of emulsion 3: (a) freshly prepared; (b) the ninth day

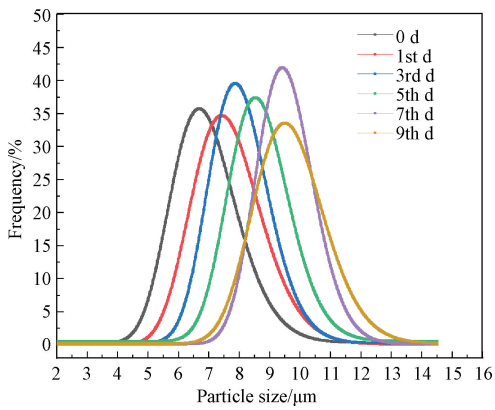


Fig. 3 Log-normal distribution of particle sizes of PFOB emulsion from freshly prepared to the ninth day

As shown in Fig. 3, the average particle size of the emulsion gradually increases over time, from an initial 6.95 μm to 9.85 μm. The peak of the particle size frequency rises from 35.76% to 41.99%, and then decreases to 33.60%. Concurrently, the curve gradually shifts to the right, indicating that the average particle size increases. It could be found that the aging mechanism of the emulsion involves coagulation followed by coalescence as reported in Refs. [19–20].

2.1.2 Oxygen solubility analysis

The oxygen solubility in emulsion 3 saturated with air was determined by the modified enzymatic method. The oxygen solubility in emulsion 3 changes with the exposure time, as shown in Fig. 4.

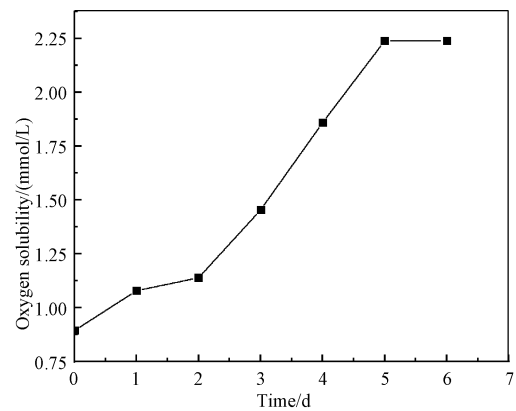


Fig. 4 Curve of oxygen solubility versus exposure time for emulsion 3

According to Fig. 4, the oxygen solubility in the emulsion gradually increases over time, reaching saturation on the fifth day. The saturation oxygen solubility in emulsion 3 is 2.240 mmol/L, which is close to the

measured value (2.130 mmol/L) by gas-liquid chromatography in Ref. [2], with the difference of about 5%. The gas-liquid chromatography has disadvantages such as high costs, sophisticated instrumentation and complicated operation. Based on the test results of various emulsions, the emulsions in this study reached saturation on the fifth day. Therefore, subsequent oxygen solubility measurements were conducted on the fifth day after emulsion preparation.

To ensure the complete reaction, the reaction time was extended. As Orange G can be stored for a long time at room temperature, it is more convenient and easier to use than the reductive coupling agent that needs to be prepared immediately before use. This simplifies the experimental procedure for the emulsion oxygen solubility measurement, enhances the color sensitivity and anti-interference ability of the enzymatic method, and reduces experimental costs.

2.2 Effect of component mass fractions on oxygen solubility

2.2.1 Effect of PFOB mass fraction on oxygen solubility

On the fifth day after the preparation of the emulsion, the oxygen solubility measurement was conducted for emulsions 1–5. The results are presented in Table 3. The measured oxygen solubility is in good agreement with the theoretical value in Ref. [2] and increases with the mass fraction of PFOB. As shown in Fig. 5, the oxygen solubility increases almost linearly with the increase in the mass fraction of PFOB.

Table 3 Measured oxygen solubility and deviation from theoretical value in Ref. [2] at different PFOB mass fractions

Emulsion	PFOB mass fraction/%	Measured oxygen solubility/(mmol/L)	Theoretical oxygen solubility/(mmol/L)	Deviation/%
1	5.4	0.600	0.640	6.25
2	10.5	1.120	1.080	3.70
3	20.0	2.240	2.130	5.16
4	28.6	2.985	2.970	0.51
5	36.3	3.545	3.795	6.59

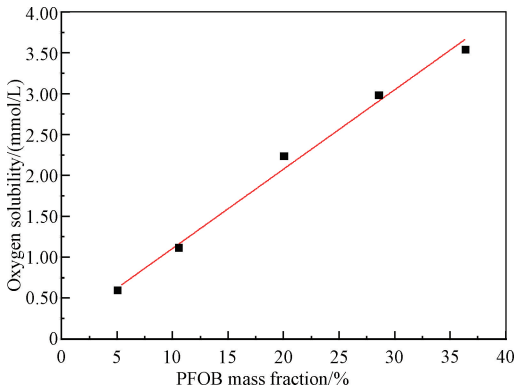


Fig. 5 Relationship between oxygen solubility and PFOB mass fraction in emulsion

2.2.2 Effect of FEO mass fraction on oxygen solubility

Emulsifiers are important components in PFC emulsions, and can maintain the emulsion stability and reduce the surface tension difference between the oil phase and the water phase. The FEO emulsifier contains perfluorinated groups, which might have a certain influence on the oxygen solubility in emulsions.

On the fifth day after the preparation of the emulsion, the oxygen solubility measurement was conducted for emulsions 3, 6 and 7, with the results being presented in Table 4.

Table 4 Measured oxygen solubility and deviation from theoretical value in Ref. [2] at different PEO mass fractions

Emulsion	FEO mass fraction/%	Measured oxygen solubility/(mmol/L)	Theoretical oxygen solubility/(mmol/L)	Deviation/%
6	5	2.055	2.130	3.52
3	10	2.240	2.130	5.16
7	15	2.240	2.130	5.16

It is indicated that the oxygen solubility in the emulsion is independent of the emulsifier mass fraction with deviations less than 8% as reported in Ref. [14]. Because the oxygen solubility in PFOB is achieved by the oxygen entering the larger intermolecular spaces of PFOB, and there are hydrogen bonds between FEO molecules, oxygen is difficult to enter the intermolecular spaces.

3 Conclusions

This study developed a modified enzymatic method by using a more stable Orange G as the color indicator to determine the oxygen solubility in the PFOB emulsion at 298.15 K and atmospheric pressure. The oxygen solubility in 0.2 mL emulsion measured by the modified

enzymatic method is close to the value by gas-liquid chromatography in the literature. The modified enzymatic method simplifies the experimental procedure of PFOB oxygen solubility measurement, enhances the color sensitivity and anti-interference ability, and reduces experimental costs. The results indicate that the oxygen solubility in the emulsions shows a positive correlation with the mass fraction of PFOB, while it is independent of the mass fraction of the emulsifier.

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全氟碳化合物乳液中氧溶解度测定的改良酶促法

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摘　要：在全氟碳化合物（perfluorocarbon，PFC）乳液中，氧溶解度是评价其在人工血液、氧气运输、诊断成像剂等领域应用性能的重要指标。然而，目前缺乏简便且准确的检测方法。本文以全氟己基乙基聚氧乙烯醚（perfluorohexylethyl polyoxyethylene ether，FEO）为乳化剂制备了全氟溴辛烷（perfluorooctyl bromide，PFOB）乳液，并开发了一种改良酶促法。该方法采用更稳定的橙黄 G 为显色指示剂来测定在 298.15 K 和大气压条件下乳液的氧溶解度。采用该改良酶促法测得 0.2 mL 乳液的氧溶解度与文献中通过气液色谱法得到的结果接近。基于该改良酶促法，进一步探究了 PFOB 和乳化剂质量分数对乳液氧溶解度的影响。结果表明，乳液氧溶解度随着 PFC 质量分数的增加而增加，而与乳化剂质量分数无显著相关性。

关键词：全氟碳化合物（PFC）乳液；氧溶解度；酶促法