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Polarographic Study on the Transportation of Oxygen by Perfluorocarbon Emulsion as Artificial Blood

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Restrictive factors of oxygen release from dispersed particles of perfluorocarbon (PFC) in a form of emulsion have been studied by polarographic reduction currents of oxygen in the emulsion. The oxygen reduction currents in PFC emulsion in open system increased linearly with the volume fraction of PFC component, and were diffusion-controlled. The increase of current was ascribed to the increase of oxygen supply to the electrode from PFC particles in the emulsion. Analyzing the oxygen reduction current due to PFC particles, the ratio of diffusion coefficients of oxygen inside and outside PFC particle (D_i/D_o) and the oxygen partition coefficient between the both sides (C_i/C_o) were obtained. The release of oxygen from PFC particle was rate-determined by oxygen diffusion in the particle and the diffusion itself was determined by inner viscosity of PFC particle. The surfactant layer of PFC particle did not play any appreciable role such as permeability barrier for oxygen transportation.

Clark and Gollan[1)] suggested that perfluorocarbons (PFC) with high oxygen solubility were useful materials for artificial blood. Later, the emulsification of PFC developed this kind of investigations,[2–5)] since bioavailability of PFC emulsion so far been demonstrated.[6–9)] However, few physicochemical studies on uptake or release of oxygen by the dispersed particles in PFC emulsion have been known,[8–10)] though relatively many kinetic studies on oxygen transport by erythrocyte were reported by different investigators.[11–14)] In order to evaluate the effectiveness of PFC emulsion as artificial blood, the limiting step of uptake or release of oxygen by PFC particle should be clarified and compared with that of erythrocyte. In addition, for better understanding of oxygen transporting capacity of erythrocyte, it may be useful that the PFC particle can be regarded as a simplified model of erythrocyte under some experimental conditions. However, spectroscopic methods, which are advantageous for erythrocytes, are inapplicable to PFC emulsion which exhibits no specific color change in uptaking or releasing oxygen. Consequently, for the estimation of oxygen binding character of such systems, electrochemical methods may be recommended.[15)] Especially polarographic techniques seem to have several advantages for PFC emulsion, further for real blood or general oxygen carriers, since the electrode *e.g.*, dropping mercury electrode (DME), to which an appropriate potential is applied, works as a model of biological surface on which oxygen is consumed immediately when it arrives at. The total limiting current due to oxygen discharge then is the value corresponding to the rate of the transport of oxygen from the bulk.

In this study, we applied d.c.-polarographic method to PFC emulsion. Then, the restrictive factors of the release of oxygen from the dispersed particles in PFC emulsion were discussed based on the behavior of the reduction currents of oxygen in PFC emulsion.

Experimental

Materials. Perfluorocarbon emulsion was a product of Green Cross Corporation (Osaka) named Fluosol-43 which is an emulsion of FC-43 (perfluorotributylamine) with a surfactant Pluronic F-68 [poly(oxypropylene)-poly(oxyethylene) copolymer, M.W. = 8350]. The average diameter of dispersed particle is 860 Å.[16)] Pluronic F-68 and FC-43 were products of Asahi Denka, Inc. (Tokyo) and Dainippon Ink and Chemicals, Inc. (Tokyo), respectively. All other reagents used were of analytical grade unless otherwise stated.

Apparatus and Procedures. Polarograph used was a Yanagimoto Polarograph Model PA 101. The dropping mercury electrode had the following characteristics: $m = 1.91$ mg s^{-1}, $t = 5.87$ s at open circuit in distilled water with 60 cm height of mercury reservoir. As for oxygen electrode, a Teflon covered gold electrode (TCGE) whose electrode surface area was *ca.* 3 mm^2, was employed for the determination of dissolved oxygen with an applied potential -0.7 *V* (*vs.* SCE). Saturated calomel electrode (SCE) was used as a reference electrode. The viscometer used was a cone and plate viscometer, made by Tokyo Keiki, Ltd. (Tokyo).

Most experiments were carried out in test solutions thermostated and under the oxygen partial pressure equilibrated with open atmosphere. For some experiments with a constant total oxygen amount in solution, a simple vessel designed as presented in Fig. 1 was used. In this vessel no gas phase is left over the liquid surface and when a given amount of test solution previously deoxygenated by nitrogen streaming, is added, the syringe moves by the increase of volume of the solution.

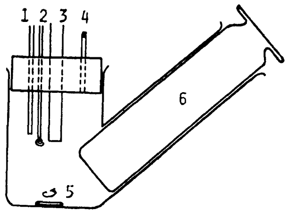

Fig. 1. Electrolytic vessel for oxygen measurement in closed system.
(1): Reference electrode, (2): working electrode, (3): indicater electrode, (4): inlet of sample solution, (5): magnetic stirrer, (6) syringe.

Results and Discussion

Effects of Pluronic F-68 on Oxygen Reduction Waves. Surfactants usually affect the electrode reaction due to the adsorption to the electrode surface.[17)] As is well known, oxygen gives two reduction waves at DME in supporting electrolyte solutions, corresponding to Eqs. 1 and 2.[18)]

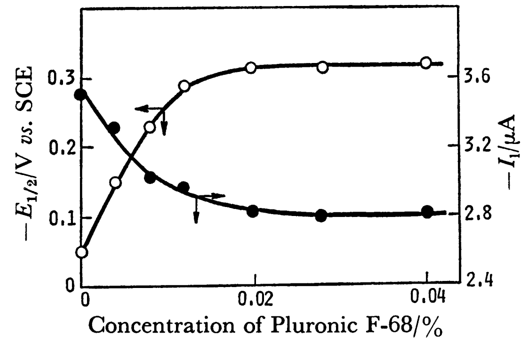

Fig. 2. Effect of Pluronic F-68 on half wave potential ($E_{1/2}$) and limiting current (I_l) of the first wave of oxygen reduction waves.
Medium: 0.1 mol dm^{-3} KNO$_3$ equilibrated with atmospheric oxygen, ○: $E_{1/2}$, ●: I_l.

$$O_2 + 2e + 2H^+ \longrightarrow H_2O_2 \qquad (1)$$

$$H_2O_2 + 2e + 2H^+ \longrightarrow 2H_2O \qquad (2)$$

Figure 2 shows the effects of Pluronic F-68 on limiting currents (I_l) and half-wave potential ($E_1/_2$) of the first wave of oxygen reduction waves. The increase of the concentration of Pluronic F-68 results in a lowering of I_l value and a shift of $E_1/_2$ to a more negative potential. Electrocapillary curves in Fig. 3 revealed the adsorption of the surfactant on the electrode surface corresponding to the decrease of I_l and the shift of $E_1/_2$ in Fig. 2. Consequently, to study the effect of the volume fraction of PFC component (ϕ) on oxygen reduction waves, experiments must be carried out without changing the concentration of Pluronic F-68. In this study, 3.2% Pluronic F-68, which was the same concentration as that in Fluosol-43, was employed. pH was maintained to be 7.4 with Tris-HCl buffer (ionic strength 0.1 mol dm^{-3}).

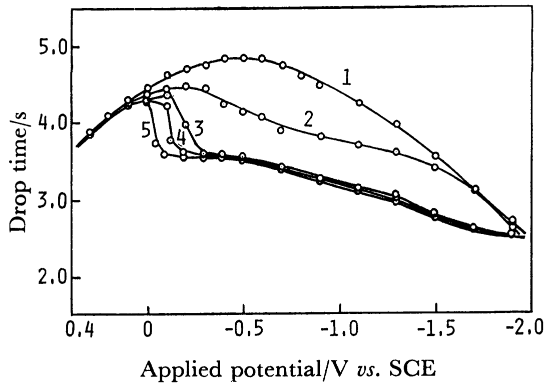

Fig. 3. Effect of Pluronic F-68 on electrocapillary curve.
Medium: the same as for Fig. 2.
Concentration of Pluronic F-68 (w/v %): (1) 0, (2) 0.004, (3) 0.008, (4) 0.016, (5) 0.04.

Oxygen Reduction Current in PFC Emulsion. The reduction current increased linearly with PFC emulsion in the electrolytic solution, when the system was open to the atmosphere (Fig. 4). Since the ratio of the 1st wave height and the 2nd did not change by adding emulsion, I_l of the 1st wave was examined in most cases. The increase in this I_l with ϕ (as seen in Fig. 4) may be attributed to the reduction of oxygen included in PFC particle, since the concentration of free oxygen in dispersion medium except PFC particles should be constant, equilibrated with atmospheric oxygen, in spite of the change of ϕ. The character of fully diffusion-controlled current was confirmed from the dependence of I_l on mercury head. Similar diffusion-controlled limiting currents of oxygen were also observed in the electrolyte solution containing 3.2% Pluronic F-68 *i.e.* the dispersion medium without PFC particles. As a consequence, the increase of current due to added PFC emulsion must also be diffusion-controlled. Then, the significance of this diffusion-controlled oxygen wave due to PFC component was considered for two possibilities: 1) Diffusion of PFC particle to the electrode and discharge of oxygen involved in PFC at the contact of the particle to the electrode: for example, Hayano and Shinozuka[19–20] reported the reduction current of a dye solubilized in surfactant micelles and concluded that the limiting step of the observed reduction current was the diffusion of surfactant micelle.

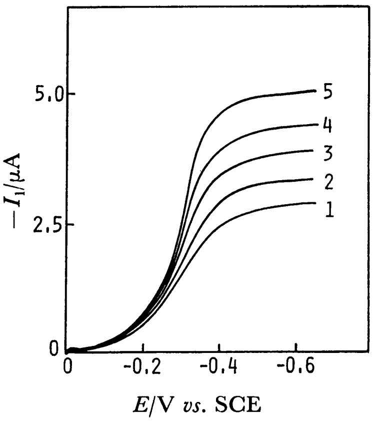

Fig. 4. Change of the oxygen reduction wave (1st wave) with volume fraction of PFC component in the electrolyte solution at 25 °C in open system.
Medium: pH 7.4, Tris-HCl buffer (ionic strength 0.1) with Pluronic F-68 3.2%. Volume fraction of PFC component (ϕ): (1) 0, (2) 0.024, (3) 0.059, (4) 0.083, (5) 0.119.

If the diffusion of the PFC particle is not adequate for the rate-determining step, other diffusion factors should be considered. 2) Direct contribution of oxygen diffusion in PFC in the particles: since our experimental conditions were sufficiently simple, the diffusion of oxygen in space other than disparsion medium would only be possible in PFC inside the dispersed particles. To simplify the treatment of problem on this assumption, we may assume that PFC particles occupy a relative space on the electrode surface, corresponding to ϕ and the remaining space ($1-\phi$) is covered by the dispersion medium.

The first assumption of the above two was examined as follows: estimating the concentration of PFC particle, its diffusion coefficient calculated from the particle size[16] and Stokes' equation, and the number of oxygen molecules in the particle, Ilkovic equation was applied to obtain the expected diffusion current due to PFC particles ($I_{\rm dem}$). The calculation† revealed that the observed diffusion current ($I_{\rm d}$) was too large to accept the first assumption. This suggests that $I_{\rm dem}$ is not determined by the diffusion of PFC particle, but by the diffusion of oxygen inside PFC particle. Present case is therefore different from that of the reduction of dye solubilized in micelle.[19–21] For PFC emulsion, it seems reasonable to consider the latter assumption of the above two.

Analysis of Oxygen Diffusion Current in Open System. If we assume that the diffusion of oxygen can be observed only in the direction perpendicular to the electrode surface, but in horizontal direction the diffusion between the particles and the medium can be neglected, the observed diffusion current ($I_{\rm d}$) may be written as

$$I_{\rm d} = I_{\rm d0,} + I_{\rm dem}, \tag{3}$$

where $I_{\rm d0,}$ and $I_{\rm dem}$ are diffusion current of free oxygen and that due to PFC particles, respectively.
Then,

$$I_{\rm d0,} = \kappa\sqrt{D_{\rm o}}C_{\rm o}\,(1-\phi), \tag{4}$$

$$I_{\rm dem} = \kappa\sqrt{D_{\rm i}}C_{\rm i}\cdot\phi, \tag{5}$$

where D and C are diffusion coefficient and concentration of oxygen, respectively and $\kappa = 607\,n\,m^{2/3}\,t^{1/6}$ (at 25 °C) with $n=2$, and ϕ is volume fraction of PFC in the system. Suffix o and i represent outside and inside of the particle, respectively. Equation 3 now can be written as

$$I_{\rm d} = \kappa\sqrt{D_{\rm o}}C_{\rm o}\,(1-\phi) + \kappa\sqrt{D_{\rm i}}C_{\rm i}\cdot\phi. \tag{6}$$

Dividing this equation by $I_{\rm d0}$

$$I_{\rm d}/I_{\rm d0} = 1 + (K\sqrt{D_{\rm i}/D_{\rm o}} - 1)\phi \tag{7}$$

can be obtained, where $I_{\rm d0}$ is $I_{\rm d}$ at $\phi=0$ ($I_{\rm d0} = \kappa\sqrt{D_{\rm o}}C_{\rm o}$)†† and $K = C_{\rm i}/C_{\rm o}$. Then, the value K should be the partition coefficient of oxygen between FC-43 and water. As seen in Fig. 5, a linear relation between $I_{\rm d}/I_{\rm d0}$ and ϕ was obtained. From the slope, $K\sqrt{D_{\rm i}/D_{\rm o}}$ was found to be 8.9±0.2. The evaluation of this value is interesting to prove the validity of the assumption presented as the possibility (2) in the above section. Namely, we must evaluate our K value involved in Eq. 7 on the basis of experimental results, and compare it with that obtained by other methods. Because no further information could be independently obtained with the open system, experiments with closed system were then carried out, in order to estimate K and $D_{\rm i}/D_{\rm o}$.

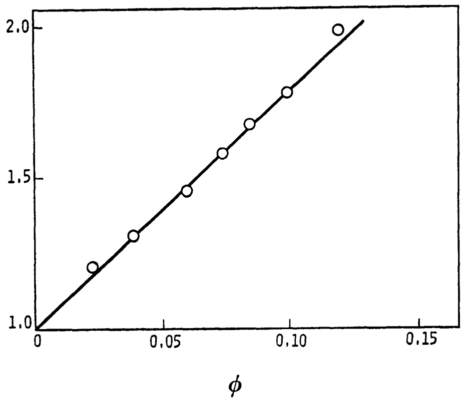

Fig. 5. Plot of $I_{\rm d}/I_{\rm d0}$ against volume fraction of PFC component (ϕ) in electrolyte solution. Explanation in text.

Evaluation of K *and* D$_{\rm i}$/D$_{\rm o}$ *with Closed System.* Using the vessel shown in Fig. 1, experiments were carried out both with TCGE and DME, but the latter enabled us more intimate analysis than TCGE. So, we introduce here the method with DME more in detail and for TCGE only some discussion will be added.

The above Eq. 6 for open system is also valid for the present closed system. However, since the absolute oxygen content in closed system is constant, the following equation can be added.

$$C_{\rm o}(1-\phi)V + C_{\rm i}\phi V = C^0V_0, \tag{8}$$

where V is the volume of the test solution closed against atmosphere and V_0 is V at $\phi=0$. C^0 is the oxygen concentration at $\phi=0$. The experiment starts with a requisite volume of electrolyte solution equilibrated with atmospheric oxygen but now closed against the atmosphere. Then, a small constant volume of deoxygenated PFC emulsion of known concentration may be added. Each $I_{\rm d}$ can be measured from its polarogram and, ϕ and V are easily calculated.
From Eqs. 6 and 8 the following relation can be derived.

$$I_{\rm d}V/(I_{\rm d0}V_0) = [1 + (K\sqrt{D_{\rm i}/D_{\rm o}} - 1)\phi]/[1 + (K-1)\phi] \tag{9}$$

Further, modifying the Eq. 9,

$$\frac{1}{1-(I_{\rm d}V)/(I_{\rm d0}V_0)} = \frac{1}{K(1-\sqrt{D_{\rm i}/D_{\rm o}})}\cdot\frac{1}{\phi} + \frac{K-1}{K(1-\sqrt{D_{\rm i}/D_{\rm o}})} \tag{10}$$

is available.

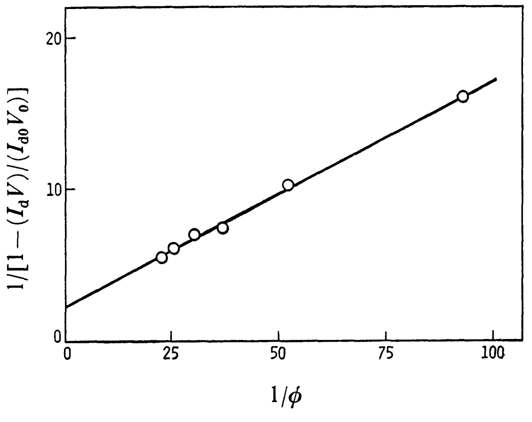

Fig. 6. Graphical treatment for estimation of partition coefficient of oxygen between inside and outside PFC particle $K = C_{\rm i}/C_{\rm o}$ and $D_{\rm i}/D_{\rm o}$ using the experimental results in closed system. Medium: the same as for Fig. 4. Explanation in text.

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† See Appendix.

†† The diffusion coefficient $D_{\rm o}$ may change with concentration of emulsion particles. However, for the sake of simplifying the treatment of problem, it was neglected for this study, since the experiments were carried out only in relatively dilute emulsion system, that is $\phi < 0.12$. Namely, $D_{\rm o}$ in emulsion was regarded to be always equal to that in dispersion medium ($\phi=0$).

As seen in Fig. 6, plot of $1/[1-(I_dV)/(I_{d0}V_0)]$ against $1/\phi$ gave a straight line. From the slope and intercept on ordinate, $K=16.4\pm0.7$ and $\sqrt{D_i/D_o}=0.61\pm0.03$ could be obtained. The D_i value was 7.4×10^{-6} cm^2 s^{-1}, assuming that D_o was 2.0×10^{-5} cm^2 s^{-1}.[†††][22,23] Consequently, $K\sqrt{D_i/D_o}=10.0$ which is not identical with the value 8.9 obtained from the experiment in open system. However, The value $\sqrt{D_i/D_o}$ is interesting, since according to Stokes' equation, D should be reciprocally proportional to the viscosity. The validity of 0.61 can be confirmed by measuring the viscosities (η) of both the dispersion medium and that of dispersed substance separately. Our measurement of η for pure FC-43 and that for the electrolyte solution containing the surfactant employed, gave a satisfactory result, *i.e.* $\sqrt{\eta_o/\eta_i}=0.57$. The reciprocal relationship between D_i/D_o and η_i/η_o suggests that the release of oxygen from PFC particles was rate-determined by the viscosity of dispersed phase, and surfactant layer of PFC particle does not play any appreciable role such as the permeability barrier for oxygen release. This favorable permeability of the surfactant layer for oxygen is not due to the dimensions of thickness of surfactant layer of PFC particle, but it may arise from the oxygen-philic property of Pluronic F-68. Because the thickness of surfactant layer of PFC particle, which can be estimated to be *ca.* 70 Å[**], is close to that of common biomembranes such as erythrocyte.[24] The K value obtained was nearly equal to the calculated one from the solubility of oxygen in FC-43[25] and in water.[26] The validity of this K value estimation was again proved by applying TCGE to the closed system as described in the following part.

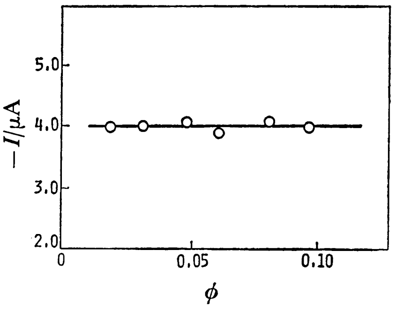

Fig. 7. Effect of PFC emulsion on reduction current of oxygen at Teflon covered gold electrode (TCGE) in open system. Medium: the same as for Fig. 4. Current was measured at −0.7 V (*vs.* SCE).

Estimation of K *by Oxygen Electrode.* Figure 7 shows that in open system the cathodic current obtained by TCGE as oxygen electrode at a fixed applied potential in a relatively low concentration range seems to be constant and proportional to the oxygen concentration in the dispersion medium, irrespective of the change of ϕ in system, while in the same PFC concentration range I_d at DME exhibits an apparent increase. The current obtained by TCGE (I) may be expressed as

$$I = \alpha C_o(1-\phi) \qquad \alpha\text{: const.} \tag{11}$$

For closed system, Eq. 8 is also valid. Then, the following equation can be derived from Eq. 8 and 11.

$$I_0V_0/(IV) = 1 + K\phi/(1-\phi), \tag{12}$$

where I_0 is I at $\phi=0$ and equal to αC_o.

With the experiments similar to those with DME in closed system, $I_0V_0/(IV)$ can be plotted against $\phi/(1-\phi)$ and K can be estimated as a slope of the obtained straight line, which intercepts the ordinate at 1.0, as shown in Fig. 8. Our result gave $K=15.3$ which should be theoretically equal to that obtained from DME. The disagreement of the values may be partly due to the difficulty of experiment in closed system. Especially the work with oxygen electrode, may require a larger caution for stability of electrode surface under the present conditions. For this reason, we recommend DME method rather than solid electrode method.

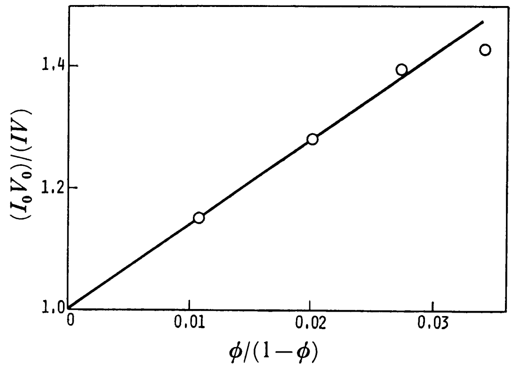

Fig. 8. Graphical treatment for the estimation of partition coefficient of oxygen between inside and outside PFC particle by TCGE in closed system. Medium: the same as for Fig. 4. Explanation in text.

Concluding Remarks. The above results may support the validity of the second possibility of the two assumptions presented. Consequently, the restrictive factor of the release of oxygen from PFC particle is the diffusion rate of oxygen inside PFC particle. The oxygen equilibrium between inside and outside of PFC particle seems to attain sufficiently fast. Further, the particle size is not the factor to change I_{dem}, as Ohyanagi and Mitsuno[8] reported that the rate of uptake and release of oxygen by PFC particle was independent of the particle size. The DME method may be expected to give some quantitive informations on the behavior of oxygen inside a larger particle such as erythrocyte. The application will be reported elsewhere.

Appendix

According to the first assumption, the diffusion current of

[†††] As diffusion coefficient of oxygen in buffer solution pH 7.4, 2.0×10^{-5} cm^2 s^{-1} was used for the present study, since several investigators have reported similar values with minor deviations for various electrolyte solution at 20—25 °C.

[**] For the calculation of this value, first we obtained volume fraction of PFC particle (ϕ_p) in the emulsion by centrifugation. This ϕ_p is comprised of both inside PFC component (ϕ) and surfactant layer component. Since the average particle diameter ($2r$) was known experimentally,[16] and ϕ of the stock emulsion was given by the manufacturer, the thickness of the surfactant layer (δ) can be readily obtained by the following equation,

$$\delta = r[1-(\phi/\phi_p)^{1/3}]$$

oxygen due to PFC particles (I_{dem}) is limited by the diffusion of PFC particle itself. Applying Ilkovic equation to I_{dem}, it can be written as

$$I_{\mathrm{dem}} = \kappa^* \sqrt{D_{\mathrm{p}}} C_{\mathrm{p}},$$
$$\kappa^* = 607 n^* m^{2/3} t^{1/6},$$

where D_{p} and n^* are the diffusion coefficient of PFC particle and the number of electrons transferred at the electrode reaction per PFC particle, respectively. Assuming all of oxygen molecules inside PFC particle might simultaneously discharge at the contact of the particle to the electrode, n^* would be given by multiplying the number of electrons transferred at the reduction of oxygen (n) by the number of oxygen molecules inside PFC particle, that is,

$$n^* = n \cdot C_{\mathrm{l}} \cdot v \cdot N,$$

where C_{l}, v, and N are concentration of oxygen dissolved in PFC, the average volume of a PFC particle and Avogadro's number, respectively. C_{p} is molar concentration of PFC particle, and can be written as

$$C_{\mathrm{p}} = \phi/(v \cdot N).$$

From the above relations, I_{dem} can be rewritten as

$$I_{\mathrm{dem}} = \kappa \sqrt{D_{\mathrm{p}}} C_{\mathrm{l}} \phi,$$
$$\kappa = 607 n m^{2/3} t^{1/6}.$$

On the other hand, the diffusion current due to free oxygen ($I_{\mathrm{dO_2}}$) can be written as

$$I_{\mathrm{dO_2}} = \kappa \sqrt{D_{\mathrm{o}}} C_{\mathrm{o}}$$

The observed diffusion current (I_{d}) is given by Eq. 3. Therefore, the ratio $I_{\mathrm{d}}/I_{\mathrm{dO_2}}$ can be written as

$$I_{\mathrm{d}}/I_{\mathrm{dO_2}} = 1 + K \sqrt{D_{\mathrm{p}}/D_{\mathrm{o}}} \cdot \phi,$$
$$K = C_{\mathrm{l}}/C_{\mathrm{o}}.$$

Now, we can estimate D_{p} in water to be $4.5 \times 10^{-8}\ \mathrm{cm^2\ s^{-1}}$ at 25 °C, using Stokes' equation, $D = RT/(N \cdot 6\pi\eta r)$, with the average diameter ($2r$) reported: 860 Å,16) and the viscosity of water as dispersion medium (η): 0.890 cp ($1\mathrm{cp} = 1 \times 10^{-3}\ \mathrm{N\ s\ m^{-2}}$) at 25 °C. Since $2.0 \times 10^{-5}\ \mathrm{cm^2\ s^{-1}}$ is taken as D_{o} value, $\sqrt{D_{\mathrm{p}}/D_{\mathrm{o}}} = 4.7 \times 10^{-2}$ can be obtained. At $\phi = 0.12$, $I_{\mathrm{d}}/I_{\mathrm{do_2}}$ value can be calculated to be 1.1 with the above obtained values, that is, the diffusion current would increase only by one tenth at this ϕ. In fact, the diffusion current observed was twice that at $\phi = 0$. This result allows us to eliminate the first assumption to explain the increase of diffusion current with ϕ.

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