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Biezen, S.A.M.; Janssen, A.P.M.; Janssen, L.J.J.

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Solubility of oxygen in glucose solutions

S.A.M. van Stroe-Biezen, A.P.M. Janssen and L.J.J. Janssen

Laboratory of Instrumental Analysis, Faculty of Chemical Technology, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (Netherlands)

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Abstract

A knowledge of the solubility of oxygen in glucose-containing solutions is essential for the determination of the kinetics of the glucose oxidase-catalysed glucose oxidation. The enzyme glucose oxidase was used in a new glucose sensor. Combination of data for the dynamic viscosity and density from the literature and data from measurements with a rotating disc electrode (RDE) for hydrogen peroxide and hydroquinone showed that the factor $\eta D$ ($\eta$ = dynamic viscosity; $D$ = diffusion coefficient) remains constant in solutions with a glucose concentration ranging from 0 to 1 M. Assuming that this is also valid for oxygen, the diffusion coefficient of oxygen in glucose solutions was calculated and the solubility of oxygen was determined with RDE measurements. At both 25 and 37°C the relationship between the solubility of oxygen and the glucose concentration is a second-degree polynomial.

Keywords: Enzymatic methods; Glucose; Oxygen; Solubility

The enzyme glucose oxidase was used in a new design for a glucose sensor. In this approach, it is necessary to know the concentration profiles of all participating compounds and to determine the exact position of hydrogen peroxide production in the sensor. If the detection electrode is situated at this location, a maximum amount of hydrogen peroxide is detected. In the sensor the enzyme is immobilized in a hydrogel. When the kinetic parameters of the immobilized enzyme are determined, the enzyme-containing gel is brought into contact with solutions with a different glucose concentration. These solutions, and also the enzyme-containing gel, are saturated with oxygen. A knowledge of the solubility of oxygen in the glucose solutions is essential, as the kinetics of the enzyme also depend on the oxygen concentration.

The glucose solutions used in these studies were prepared with phosphate-buffered saline (0.050 M NaH$_2$PO$_4$, 0.050 M Na$_2$HPO$_4$ and 0.16 M NaCl, pH 7). The enzyme glucose oxidase (GOD) catalyses the oxidation of glucose:

$$\text{glucose} + \text{GOD}_\text{ox} \rightarrow \text{gluconolactone} + \text{GOD}_\text{red}$$

$$\text{O}_2 + \text{GOD}_\text{red} \rightarrow \text{H}_2\text{O}_2 + \text{GOD}_\text{ox}$$

(1)

The rate of production of hydrogen peroxide ($v$) is given by [1]

$$\frac{1}{v} = \frac{1}{v_{\text{max}}} \left(1 + \frac{k_0}{C_0} + \frac{k_g}{C_g}\right)$$

(2)

where $v_{\text{max}}$ is the maximum reaction rate (mol m$^{-3}$ s$^{-1}$), $k_0$ and $k_g$ are the Michaelis-Menten constants (mol m$^{-3}$) for oxygen and glucose, respectively, and $C_0$ and $C_g$ are the concentrations of oxygen and glucose (mol m$^{-3}$), respectively. To determine the kinetic parameters $v_{\text{max}}$, $k_0$ and $k_g$ from the production rate of hydrogen peroxide...
(\(\nu\)), the concentrations of oxygen and glucose must be known.

**THEORY**

The determination of the solubility of oxygen can be carried out by using a rotating disc electrode (RDE). The well known Levich relationship is used for RDE experiments [2]:

\[
I_{\text{lim}} = 0.62nFA_e C D^{2/3} \nu^{-1/6} \omega^{1/2}
\]

where \(I_{\text{lim}}\) is the limiting current (A), \(n\) the number of electrons involved in the electrode reaction, \(F\) the Faraday constant, i.e., the charge on 1 mol of electrons (A), \(A_e\) the electrode area (m²), \(C\) the bulk concentration of the electroactive species (mol m⁻³), \(D\) the diffusion coefficient of the electroactive species (m² s⁻¹), \(\nu\) the kinematic viscosity of the solution (m² s⁻¹) and \(\omega\) the angular rotation speed (rad s⁻¹). \(D\) and \(\nu\) will alter as a result of changes in glucose concentration.

To solve this problem, a Stokes–Einstein-type relationship is used [3]:

\[
\eta D = \text{constant} = B
\]

where \(\eta\) is the dynamic viscosity of the solution (kg m⁻¹ s⁻¹). From data for the dynamic viscosity and the density (\(\rho\)), the kinematic viscosity \(\nu\) (\(= \eta / \rho\)) as a function of the glucose concentration was obtained. As the diffusion coefficient of oxygen in the absence of glucose is well known, it is possible to determine the diffusion coefficient of oxygen for several glucose concentrations from Eqn. 4. Equation 3 can be applied to calculate the solubility of oxygen.

The validity of Eqn. 4 was checked for two electroactive species, viz., hydrogen peroxide and hydroquinone, because for these species the concentrations in the glucose solution are chosen. However, this is not the case for oxygen.

**EXPERIMENTAL**

**Reagents**

Phosphate-buffered saline (PBS) was prepared with \(\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}, \text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}\) and \(\text{NaCl}\) purchased from Merck. Hydrogen peroxide [30% (w/w), aqueous solution] was obtained from Chempro Pack, hydroquinone from Merck and D-glucose from Janssen Chimica. Platinum black electrodes were prepared with a solution of \(\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}\) from H. Drijfhout and \(\text{PbCl}_2\) from Merck. All solutions were prepared with demineralized, distilled water.

**Instrumentation**

For the RDE experiments, a Wenking POS 73 potentiostat was used, equipped with a digital multimeter (Fluke 8600 A) and a Motomatic E-550-M stirring motor. Recording was carried out with either an \(x-\gamma\) recorder (Philips 8120) for polished platinum RDE cyclic voltammograms or an \(x-t\) recorder (Kipp BD40) for platinum black RDE experiments. A circulating water-bath (Colora NB-32981) was used for temperature control of the one-compartment cell.

For preparation of the platinum black electrodes, a Delta Elektronika E030-1 power supply was used, connected with a sliding resistance (Albert van der Perk) and an amperometer (Gossen).

**Preparation of a platinum black RDE**

A polished platinum RDE was scanned from \(-1500\) to \(+1500\) mV (vs. SCE) with a scan rate of \(1 \text{ V s}^{-1}\) in a \(2 \text{ M H}_2\text{SO}_4\) solution to remove all impurities. The electrode was immersed in \(3\%\) (w/w) \(\text{H}_2\text{PtCl}_6\) solution [containing \(0.02\%\) (w/w) \(\text{PbCl}_2\)] and connected as the cathode with a platinum sheet as the anode. A current of about \(5 \text{ mA}\) was used to prepare a platinum black layer on the platinum RDE within 10 min. Subsequently the platinum black electrode (platinized electrode) was washed in running tap water for at least 30 min and then washed with distilled, demineralized water for 5 min.

**Procedures**

For all experiments a platinum RDE (polished or platinized) was used as the working electrode \((A_e = 0.50 \times 10^{-4} \text{ m}^2)\). Further, a platinum counter electrode with a surface area of \(5 \times 10^{-4} \text{ m}^2\) and a saturated calomel reference electrode (SCE) with a Luggin capillary were placed in the
one-compartment cell. A circulating water-bath was used to keep the temperature constant. As supporting electrolyte PBS (0.050 M NaH₂PO₄, 0.050 M Na₂HPO₄ and 0.16 M NaCl, pH 7) was used. The glucose concentrations in this electrolyte varied from 0 to 1.0 M.

Hydroquinone experiments (2–3 mol m⁻³) were performed with an argon-saturated (1 atm) glucose solution. Hydroquinone was added before passing argon through. A cyclic voltammogram was recorded from −550 to +1200 mV (vs. SCE) at various rotation rates (1–9 rps). Rotation rates were varied in random order. A scan rate of 50 mV s⁻¹ was used. After every set of measurements belonging to one glucose concentration the RDE was cleaned by scanning from −1500 to +1500 mV (vs. SCE) in 2 M H₂SO₄ with a scan rate of 1 V s⁻¹.

For hydrogen peroxide measurements (3–4 mol m⁻³) the glucose solution was saturated with argon (1 atm) before adding hydrogen peroxide and cyclic voltammograms were scanned cathodically from +300 to −750 mV (vs. SCE) at 50 mV s⁻¹. Again the rotation speeds were varied in random order and the RDE was cleaned in 2 M H₂SO₄ after each set of measurements. Hydrogen peroxide diffusion coefficients were also determined by using a platinized RDE. An oxidation potential of +700 mV vs. SCE was applied and the electrode was allowed to reach a steady background current for a glucose solution without hydrogen peroxide at a certain rotation speed. Thereafter an aliquot of a hydrogen peroxide stock solution was added while leaving the potential at +700 mV. The solution was stirred magnetically for a few seconds to make it homogeneous and a steady current was obtained.

Oxygen measurements were carried out with a platinum black electrode. Glucose solutions were saturated with argon to determine the background current at −580 mV vs. SCE at a certain rotation speed. Subsequently the solution was saturated with oxygen (1 atm) while leaving all other conditions unchanged. After about 10 min a steady reduction current could be measured. Measurements for all three compounds were made at 25 and 37°C. The temperature was controlled with a circulating water-bath.

RESULTS

From the dynamic viscosity η [4] and the density ρ [5] as a function of the weight percentage of glucose, the dynamic viscosity was calculated as a function of the molar glucose concentration (Fig. 1), taking into account the influence of NaCl, NaH₂PO₄ and Na₂HPO₄. If mutual interactions between the salt ions and glucose are neglected, the Grunberg–Nissan relationship can be used [6]:

\[
\log \eta_a = x_w \log \eta_w + x_1 \log \eta_1 + x_2 \log \eta_2 + x_3 \log \eta_3
\]

where \(x_w\) is the mole fraction of water, \(x_i\) is the mole fraction of compound \(i\), \(\eta_a\) and \(\eta_w\) are the dynamic viscosity of the solution and pure water, respectively, and \(\eta_i\) is the apparent dynamic viscosity of compound \(i\). Further,

\[
\log \eta_a = x_{w,a} \log \eta_w + x_{1,a} \log \eta_1
\]

\[
\log \eta_b = x_{w,b} \log \eta_w + x_{2,b} \log \eta_2
\]

\[
\log \eta_c = x_{w,c} \log \eta_w + x_{3,c} \log \eta_3
\]

where the subscripts \(a\), \(b\) and \(c\) refer to aqueous solutions of NaCl, sodium phosphate and glucose, respectively. When \(x_i\) is assumed to be equal in

![Fig. 1. Dynamic viscosity (■ = 25°C; + = 37°C) and density (▲ = 25°C; ○ = 37°C) as a function of the glucose concentration in PBS.](image)
Eqn. 5 and in Eqns. 6–8 (viz., \(x_{1,a} = x_1; x_{2,b} = x_2; x_{3,c} = x_3\)) it follows that
\[
\begin{align*}
  x_w + x_1 + x_2 + x_3 &= 1 \quad \text{(from Eqn. 5)} \\
  x_{w,a} + x_1 &= 1 \quad \text{(from Eqn. 6)} \\
  x_{w,b} + x_2 &= 1 \quad \text{(from Eqn. 7)} \\
  x_{w,c} + x_3 &= 1 \quad \text{(from Eqn. 8)}
\end{align*}
\]

From this set of equations, the following can easily be derived:
\[
x_w - x_{w,a} - x_{w,b} - x_{w,c} = -2 \quad \text{(10)}
\]

Combining Eqns. 5–8 and 10 gives
\[
\eta_a = \eta_a \eta_b \eta_c / \eta_w^2 \quad \text{(11)}
\]

Values for \(\eta_w, \eta_a, \eta_b, \text{and } \eta_c\) can be found in the literature [4,7]. The same procedure can be followed for calculating the density for the glucose–PBS solutions [5,8].

Cyclic voltammograms of hydrogen peroxide and hydroquinone in the glucose solutions have similar shapes to those in pure PBS. Plots of \(I_{\text{lim}}\) versus \(\omega^{1/2}\) give straight lines although at higher rotation rates a small deviation is observed owing to kinetic limitations. Therefore, a reciprocal plot is made, which is linear even for high rotation rates. If, however, the original \(I_{\text{lim}}\) versus \(\omega^{1/2}\) plot has an intercept \((I^*)\), a correction should be performed by subtracting \(I^*\) from all measured limiting currents. Only from these corrected values can a proper reciprocal plot be obtained (see Figs. 2 and 3).

Contamination of the polished platinum electrodes easily occurs, especially in solutions with a high glucose concentration \((\geq 0.5\,\text{M})\). Cleaning of the electrodes in sulphuric acid was necessary after every set of measurements (1–9 rps) for one glucose concentration. If measurements were performed going from a low to a high rotation speed, the Levich slope was different from measurements made going from a high to a low rotation speed (Fig. 4). This effect was not observed in a glucose-free solution (Fig. 4). The average of the two slopes, however, gave good results. A random order of measuring while alternating low and high rotation speeds yielded the same results. This is shown in Fig. 5 for hydroquinone and hydrogen peroxide.

![Fig. 2. Current as a function of square root of the angular rotation rate for 2 mM hydroquinone in PBS at 25°C. The continuous line follows the measured curve and the dashed line is the linear curve for rotation speeds from 1 to 5 rps. \(I^*\) denotes the intercept.](image)

![Fig. 3. Reciprocal of the corrected current \([(I-I^*)^{-1}]\) as a function of the reciprocal of the square root of the angular rotation rate for 2 mM hydroquinone in PBS at 25°C.](image)
Fig. 4. Current plotted against square root of the angular rotation rate for 2 mM hydroquinone at 25°C. Measurements in PBS with (○) rising and (■) falling rotation speeds give the same results. For PBS containing 1 M glucose measurements with (○) rising or (△) falling rotation speeds do not match.

is not possible to obtain a voltammogram showing a limiting current, even if a low scan rate of 1 mV s⁻¹ is used. Therefore, a potential at which a limiting current appears was directly applied. For a hydrogen peroxide-free medium it took 1–3 h (depending on the glucose concentration) to reach a background current at +700 mV. The results for the platinum black electrode were consistent with those for the polished platinum electrode. The advantage of the platinum black electrode is that the limiting current remains constant for at least 15 min.

Figure 6 shows the factor $\eta D$ for both hydrogen peroxide and hydroquinone as a function of glucose concentration. It can be clearly seen that within the examined range of glucose concentrations $\eta D$ does not change significantly. Therefore, it is assumed that $\eta D$ for oxygen does not depend on the glucose concentration either. It is known that oxygen, hydrogen peroxide and hydroquinone behave similarly when diffusing through a hydrogel layer [9]. Further, it is known that the Stokes–Einstein relationship is valid for oxygen in NaCl solutions [10].

As the diffusion coefficient of oxygen in PBS is measured as $1.94 \times 10^{-9}$ m² s⁻¹ [9] and $\eta$ for this solution is known to be $0.938 \times 10^{-3}$ kg m⁻¹ s⁻¹ (Fig. 1), $\eta D$ can be calculated as $1.82 \times 10^{-12}$
kg m s\(^{-2}\). At 37°C, \(\eta D\) becomes \((2.47 \times 10^{-9}) \times (0.728 \times 10^{-3}) = 1.80 \times 10^{-12}\) kg m s\(^{-2}\). From these values for \(\eta D\) and the values for \(\eta\) (Fig. 1), the diffusion coefficient of oxygen was calculated as a function of the glucose concentration (Fig. 7). With a platinum black electrode the solubility of oxygen in glucose solutions was determined, using the diffusion coefficients from Fig. 7. The results are presented in Fig. 8 for 25 and 37°C.

The solubility of oxygen is likely to be slightly changed by the presence of salt ions (salting out effect [11]). The solubility of oxygen as a function of the glucose concentration at 25 and 37°C is given by

\[
C_o = 1.045 - 0.2687C_g + 0.09714C_g^2 \quad (12)
\]

\[
C_o = 0.8607 - 0.1689C_g + 0.04571C_g^2 \quad (13)
\]

respectively. These fitted relationships are applicable to a glucose concentration range of 0–1 M. The deviation between the measured values and values calculated with Eqn. 12 or 13 is \(\leq 0.3\%\).

References