J BIOLUMIN CHEMILUMIN 1998; 13: 125–130

Dissolved Oxygen Determination by Electrocatalysed Chemiluminescence with In-line Solid Phase Media

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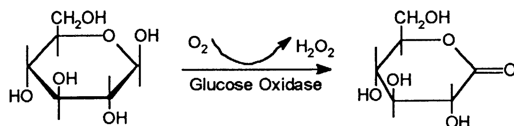
Dissolved elemental oxygen is determined in a flowing aqueous stream using glucose oxidase to catalyse the reaction between D-glucose and O_2 to produce hydrogen peroxide. The levels of the resulting H_2O_2 are detected and quantified by luminol chemiluminescence using in-line solid phase media for pH adjustment of the reagent stream and for controlled release of the luminophore. The reaction is initiated by electrochemical catalysis. By the use of excess D-glucose in the reagent flow stream, the intensity of chemiluminescence is rendered proportional only to fluctuations in the dissolved O_2 concentration. The methodology provides a means for the detection of aqueous O_2 in the range 0–10 mg/L. © 1998 John Wiley & Sons, Ltd.

Keywords: chemiluminescence; electrocatalysed; luminol; oxygen; glucose

Received 15 July 1997; revised 4 November 1997; accepted 17 November 1997

INTRODUCTION

Glucose oxidase catalyses the oxidation of β-D-glucopyranose to D-glucono-δ-lactone by molecular oxygen:



In this reaction H_2O_2 is produced on a 1:1 molar basis. The glucose oxidase mediated production of hydrogen peroxide has been employed in a variety of amperometric, fluorometric and chemiluminescence (CL) methodologies for the detection and quantitation of D-glucose (1,2–6). The use of this reaction for the determination of oxygen has received far less attention (3,7,8).

For the purposes of oxygen determination by luminol chemiluminescence in a flow analysis configuration, the hydrogen peroxide production reaction is confined within a packed bed of glucose oxidase immobilized on an inert support. The flowing aqueous D-glucose stream containing the sample passes through the glucose oxidase bed, where the production of hydrogen peroxide is proportional to the oxygen concentration. Conditions required to promote CL detection are achieved by flow of an aqueous reagent carrier stream through a series of in-line modules containing crystalline media. The solid phase basification (SPB) module contains crystalline magnesium oxide and produces an effluent $pH > 10.5$. The basified effluent from the SPB module flows into the solid phase luminophore (SPL) module consisting of a packed bed of sublimation-recrystallized luminol. The SPL module

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Contract/grant sponsor: NASA, Lyndon B. Johnson Space Center, Houston, TX, USA; Contract/grant number: NAS9-19305.

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CCC 0884–3996/98/030125–06 $17.50

releases the luminophore to the aqueous stream at a controlled rate which is governed by the alkalinity of the reagent carrier stream flowing into the module (9). By controlling the pH, near constant levels of luminophore addition ($\approx$ 50 mg/L) can be sustained until the media are almost totally depleted. During luminol dissolution, the pH of the carrier stream drops significantly. For this reason, a second SPB module is used to re-adjust the pH of the effluent from the solid phase luminol (SPL) module to the alkaline pH required for the reaction. The use of SPB and SPL media has been reported previously for the flow analysis determination of hydrogen peroxide (9) and ethanol (10). The carrier stream and the H_2O_2-containing sample stream mix at the inlet to the photodetection cell. The chemiluminescent reaction between H_2O_2 and luminol is catalysed by an electric field between gold electrodes (11–14) and results in the emission of photons with maximum intensity at = 425 nm (15). Electrocatalysis provides an effective means for initiation of the luminescent reaction within the photodetection cell.

EXPERIMENTAL

Chemicals and materials

Glucose oxidase (glucose oxygen oxidoreductase, EC 1.1.3.4), horseradish peroxidase (EC 1.11.1.7), O-dianisidine, and ethylene diamine were purchased from Sigma (St. Louis, MO). Luminol (3-amino-phthalhydrazide), D-glucose, glutaric dialdehyde (glutaraldehyde), sodium bicarbonate, hydrochloric acid, dibasic sodium phosphate, monobasic potassium phosphate, and sodium acetate were purchase from Aldrich (Milwaukee, WI). Titanium tetrachloride, carbon tetrachloride, 30% hydrogen peroxide, and copper sulphate, were purchased from VWR Scientific (Portland, OR). A Celite Bio-Catalyst Carrier R-648 (diatomaceous earth) was purchased from Manville (Denver, CO). Breathing quality compressed oxygen was purchased from Pacific Airgas (Portland, OR). 1 MΩ deionized (DI) water was prepared using mixed ion exchange beds and carbon adsorption (Culligan).

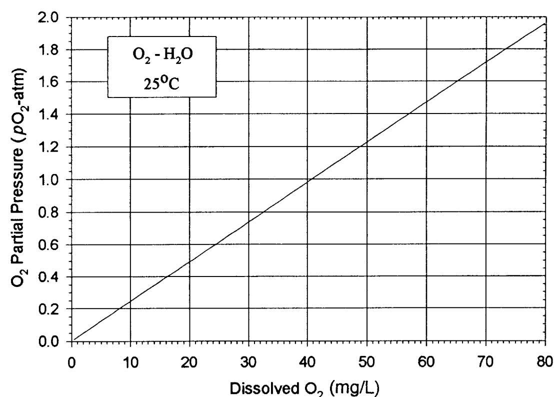

Figure 1. Henry's law relation for dissolved O_2 vs. pO_2 at 25°C

Solid phase media

Solid phase basification (SPB) beds were prepared in borosilicate glass tubes using 75–106 μm magnesium oxide crystals. Crystalline luminol was prepared by sublimation recrystallization and then packed into solid phase luminophore (SPL) beds confined within borosilicate glass tubes. Methods for the preparation of SPB and SPL beds have been described in greater detail elsewhere (9).

Enzyme immobilization procedures

Glucose oxidase was immobilized on a diatomaceous earth support using the titanium activation procedure. A 15% solution of titanium oxychloride ($TiOCl_2$) was prepared by slowly adding titanium tetrachloride into a 10% hydrochloric acid solution in an ice bath at 5°C. A 40 g portion of R-648 diatomaceous earth support was added to 100 mL of 15% $TiOCl_2$ solution, which was then evaporated to dryness under vacuum at 45°C. The dried titanium activated support was washed four times with 100 mL portions of methanol and dried at room temperature. The activated support (40 g) was combined with 100 mL of 5% ethylene diamine in carbon tetrachloride, heated at 45°C for two hours, then washed twice each with 100 mL portions of methanol and DI water. A 125 mL aliquot of 5% glutaraldehyde in phosphate buffer (pH = 8.5) was added to the support. The resulting suspension was rolled overnight. The support was then washed five times with 200 mL portions of DI water, and dried at room temperature. 10,000 EU of glucose oxidase was combined with 10 g of derivatized support in 20 mL of acetate buffer (pH = 4). The mixture was rolled for 144 hours at room temperature for the formation of the Schiff base between free amino groups of the enzyme and the free aldehyde groups of the tethered dialdehyde,

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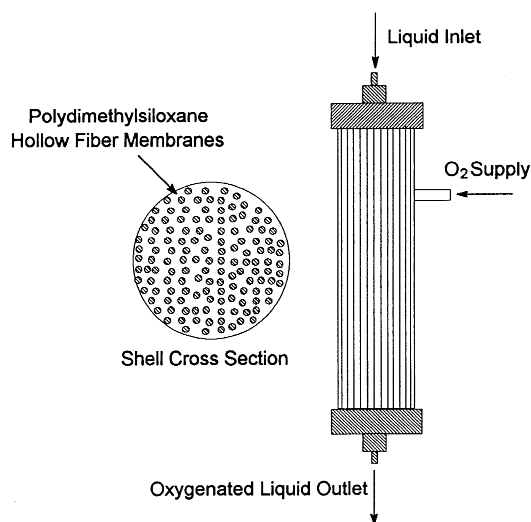

Figure 2. Tube-in-shell hollow fibre membrane oxygenator schematic

resulting in covalent binding of the enzyme to the support. The enzyme-coated support was separated from the reaction mixture by decantation. The preparation was stored in 30 mL of pH 4 acetate buffer until use.

Apparatus

Light detection was achieved using a ten dynode Hamamatsu R878, 5.08 cm diameter head-on type photomultiplier tube (PMT) with optimal spectral response at 420 nm, and a Nucleus TB-1 photomultiplier tube base. High voltage ($\approx$ 1,000 V) was applied to the PMT using a Nucleus model 575 scaler-ratemeter power supply. Conditioned PMT output was monitored using a Linear model 2030 chart recorder. Flows were established using Masterflex model 7520–35 multichannel peristaltic pumps, and a Cole-Parmer model 74900–20 dual channel syringe pump.

Electrocatalysed chemiluminescence (ECL) cell

The ECL cell was constructed from two 0.48 cm thick circular plates of 5.08 cm diameter polycarbonate. Attached to the inside of the front plate was a 2.54 cm square gold mesh electrode (Bioanalytical 94332) and a gold contact wire. The Hamamatsu R878 head-on type 5.08 cm photomultiplier tube (PMT) was optically coupled to the external surface of the front plate. A 25 mm-square by 1 mm-thick gold foil electrode (Alfa 14721) with gold contact wire was attached to the inside of the back plate. A 0.038 cm-thick PTFE gasket with a tortuous flow path was used as a spacer to separate the plates. A Microelectrodes model MI 402 Ag/AgCl reference microelectrode was installed to monitor the ECL outflow. Electrode potentials were controlled using a variable DC power supply. A polarity switching circuit was used to maintain clean electrode surfaces and to reduce cell polarization. A more detailed description of the ECL cell and support circuitry has been provided in a previous publication (9).

Hollow fiber membrane oxygenator

A membrane oxygenator was used for the production of known dissolved O_2 concentrations for use as standards. The equilibrium between atmospheric and dissolved aqueous O_2 levels is described by Henry's Law:

$$k = \frac{pO_2}{\chi}$$

where k is the Henry's Law constant, pO_2 is the atmospheric partial pressure of oxygen in atmospheres, and χ is the mole fraction of dissolved oxygen. The value of k as a function of absolute temperature (°K) is given by Benson et al. (16) as:

$$\ln k = 3.71814 + 5596.17°\mathrm{K}^{-1} - 1049668°\mathrm{K}^{-2}$$

This relation, with aqueous O_2 in units of mg/L, is presented in Fig. 1 for 25°C.

To produce the desired concentration of aqueous O_2, the flowing water stream is saturated using a membrane contactor consisting of a bundle of non-porous hollow polydimethylsiloxane membrane fibres in the tube-in-shell arrangement, as illustrated in Fig. 2. The hollow fibres are attached to the manifold at the inlet and outlet faces by an epoxy potting compound. Water flows through the hollow fibres and O_2 equilibrates across the semipermeable membranes. This arrangement facilitates mass transfer by maximization of the available surface area and minimization of diffusion distances (17, 18). The desired dissolved O_2 concentration is subsequently produced by dilution with a degassed D-glucose solution. The oxygenation device uses a bundle of 50

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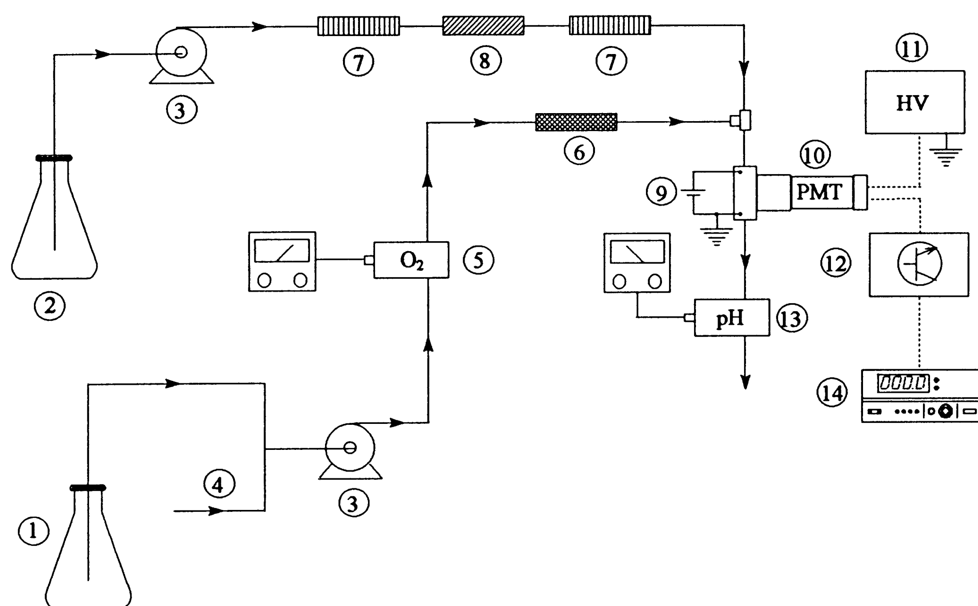

Figure 3. O_2 chemiluminescence (CL) experimental apparatus: (1) D-glucose solution; (2) DI water; (3) pump; (4) sample inlet; (5) dissolved O_2 probe; (6) glucose oxidase bed; (7) MgO bed; (8) luminol bed; (9) CL cell; (10) PMT; (11) power supply; (12) signal conditioning; (13) pH probe; (14) digital voltmeter

non-porous polydimethylsiloxane hollow fibres (0.031 cm i.d. 0.064 cm OD) inside a cylindrical polycarbonate shell, 26.7 cm in length and 2.54 cm in diameter. The liquid filled internal volume of the unit is 4.8 cm^3 and the active surface area is 124 cm^2. Dissolved O_2 levels produced by the membrane contactor were confirmed using an in-line Clark electrode type amperometric oxygen monitor (Ingold Type 170).

Integrated experimental apparatus

The apparatus used for the flow analysis determination of dissolved oxygen is illustrated schematically in Fig. 3. A peristaltic pump is used to establish reagent carrier stream flow. Sample stream delivery to the electrocatalysed luminescent (ECL) cell is accomplished using a syringe pump. The carrier stream consists of degassed deionized water which sequentially flows through a 2.5 cm^3 SPB bed, a 0.5 cm^3 crystallized luminol bed and a second 2.5 cm^3 SPB bed, each contained within a 0.3 cm i.d. borosilicate glass tube and confined by glass wool end plugs. The resultant carrier stream contains $\approx$ 50 mg/L luminol at pH $\approx$ 10.3.

The sample stream flows through a 1.0 cm^3 bed of immobilized glucose oxidase (GO) confined within a a 0.3 cm i.d. 15 cm long borosilicate glass tube with glass wool end plugs. The carrier stream and the sample stream are mixed at the inlet to the ECL cell and photodetection system. System output consists of a voltage, indicated by a digital multimeter.

RESULTS AND DISCUSSION

An initial experiment was performed to assess the adequacy of the glucose oxidase enzyme and of the enzyme immobilization procedure for the quantitative production of H_2O_2 in relation to varying influent levels of dissolved O_2. A 35 cm^3 packed bed of glucose oxidase immobilized on diatomaceous earth was used. The fractional void volume of the packed bed was approximately 50%. Assuming plug flow, this corresponds to a residence time of 8.75 min at the 2.0 cm^3/min flow rate. The enzyme bed was challenged with a blank and three solutions containing dissolved oxygen levels of 8.0, 21.9 and 26.5 mg/L. The composition of the DI water carrier stream was held at a constant 500 mg/L D-glucose concentration, corresponding to a stoichiometric excess of at least 3.3. The experimental results are given in Fig. 4. The observed H_2O_2 concentrations were very close to the

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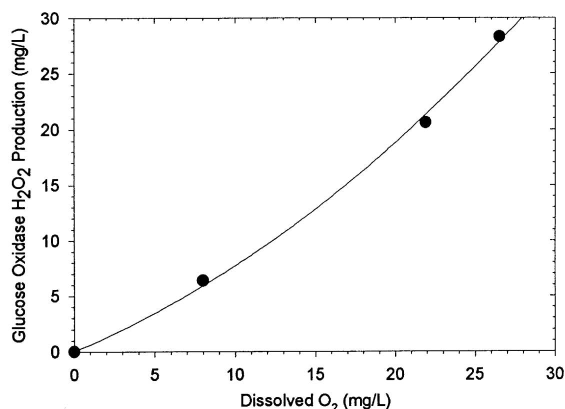

Figure 4. H_2O_2 production from glucose oxidase vs. oxygen concentration

values corresponding to 100% conversion. These data suggest that the decomposition of hydrogen peroxide within the immobilized GO bed is not significant. The data also indicate that the reaction is not limited by mass transfer within the packed bed.

To confirm the operation of the CL cell, photodetector and associated circuitry, luminol chemiluminescence-based detection of O_2 was demonstrated using the GO bed with aqueous phase reagents and chemical catalysis. Three separate flowing streams, carrying 0.4 cm^3/min alkaline luminol, 0.2 cm^3/min Cu^{+2} catalyst, and the sample stream flowing at 0.2 cm^3/min, were mixed at the inlet to the chemiluminescence cell. The adequacy of the reaction chemistry using these flow rates, and correct operation of the photodetection system, were confirmed initially using aqueous H_2O_2 in DI water sample solutions which bypassed the GO bed. CL-based oxygen detection was then initiated using DI water containing a constant D-glucose concentration of 500 mg/L. A blank and two standard solutions containing 13.3 and 26.5 mg/L dissolved O_2 were analysed. The experimental results are shown in Fig. 5.

Using the fully integrated apparatus illustrated in Fig. 3, aqueous phase reagents were minimized by the use of flow-through modules containing crystalline materials for basification and addition of the luminophore, and by the initiation of the CL reaction by electrocatalysis. The ECL cell was operated by application of a non-referenced potential of +0.8 V to the gold foil electrode. The sample stream consisted of 300 mg/L D-glucose in DI water, corresponding to a minimum fivefold stoichiometric excess. The reagent carrier stream and sample streams were operated at flow rates of 2.0 and 0.2 cm^3/min respectively. A blank and samples containing 2.2, 4.2, 5.8 and 9.8 mg/L O_2 in DI water were analysed. The results are presented in Fig. 6. The linear relationship between dissolved O_2 concentrations of 0–10 mg/L and chemiluminescent response (mV) is approximated by $[O_2]$ mg/L = 0.0747 (mV) + 0.0676, with a correlation coefficient (r^2) of 0.9958.

The determination of dissolved oxygen, using the electrocatalysed chemiluminescence-based enzymatic flow analysis methodology with in-line crystalline media for pH adjustment and controlled release

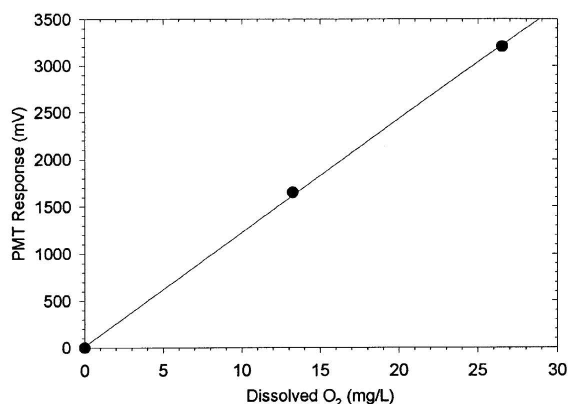

Figure 5. CL response vs. O_2 concentration for aqueous reagents

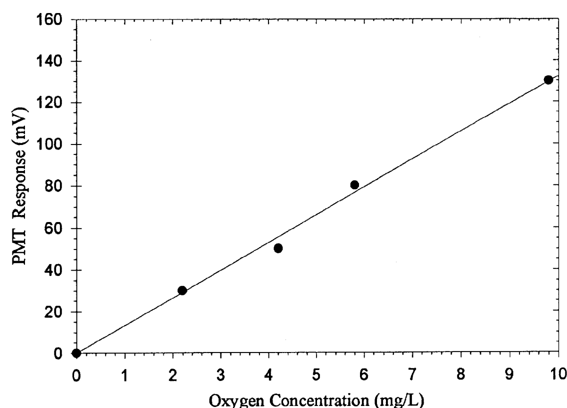

Figure 6. ECL detection and quantitation of dissolved O_2 with in-line media

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of luminol, has been demonstrated. Linear response was obtained between 0 and 10 mg/L. This span represents the concentration range obtainable for samples at room temperature and under normal atmospheric pressure conditions. Potential applications include the determination of dissolved oxygen partial pressures and biochemical oxygen demand (BOD) in natural waters and waste water. The use of in-line modules containing solid phase media to perform the functions which otherwise would require the preparation of aqueous reagents offers obvious operational advantages for flow analysis methodologies. Further work is required to clarify the SPB, SPL and GO bed size requirements in relation to flow rate, so that modules can be designed to function for specific operational time periods.

Acknowledgment

This work was supported by the National Aeronautics and Space Administration's Lyndon B. Johnson Space Center, Houston, Texas, USA, under contract NAS9-19305.

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