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Short communication

Gas-phase optrode measurements of oxygen in calorimetric vessels

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ABSTRACT

Simultaneous measurements of oxygen and heat rates are useful for the determination of thermodynamics and kinetics of metabolic and chemical reactions involving oxygen. Optrodes have not previously been used to measure oxygen inside calorimetric vessels. The optrode used in this study produces $<2\ \mu W$ and can be used to make measurements in both gaseous and aqueous systems. An external syringe injection of air was used to calibrate the optrode, and zinc–air batteries were used to evaluate the system. Data on maple leaf buds were collected to demonstrate application to respiration measurements.

1. Introduction

Simultaneous measurements of heat and oxygen rates provide a useful method to investigate chemical and biological processes. Heat rate data alone are often not sufficient to draw conclusions about complex systems, and measuring oxygen production or uptake rates simultaneously with heat rates can help elucidate the reactions occurring. Simultaneous measurement of heat rates together with oxygen rates measured with a polarographic oxygen sensor in a calorimeter has previously been reported [1–6]. However, this previous system is limited to measurements in liquid water; polarographic oxygen sensors and oxygen electrodes perform very poorly in the gas phase. Zinc–air batteries can also be used to measure oxygen partial pressure, but are not as sensitive as optrodes and produce too much heat to be used conveniently in calorimetric vessels. Pressure transducers have previously been used to measure oxygen uptake rates in calorimetric vessels [7,8], but are difficult to maintain because of the condensation of vapor that can damage the sensor and plug the connection to the calorimetric vessel. Optrodes function equally well in liquid and gas phases, but have not previously been used in conjunction with calorimetric measurements. Optrodes are constructed by attaching a fluorescent material to the end of an optic fiber, and function as sensors for an analyte that binds reversibly and quenches the fluorescence. The analyte concentration or partial pressure is measured by the change in fluorescence intensity of the flurophore bound to the tip of the optic fiber.

Optrodes, as well as these other sensors, measure the partial pressure of oxygen in the vessel. To obtain the rate of oxygen reaction, it is necessary that the vessel be sealed and to measure the rate of change in the signal over time. Converting the measured change in the signal to moles of oxygen per unit time requires knowing the volume of gas in the calorimetric vessel, a difficult quantity to estimate with variable shapes and sizes of samples. The procedure described in this paper avoids this difficulty by calibration of the sensor with the sample in situ.

2. Methods and materials

A CSC model 4100 Multi-Cell Differential Scanning Calorimeter (MC-DSC, Calorimetry Sciences Corporation, Lindon, UT) with 1 mL, hastelloy ampules was used in isothermal mode to simultaneously measure the heat rates from three samples. Heat rates were logged at 2-min intervals. A FOXY oxygen optrode system (Ocean Optics Inc., Dunedin, FL) was used to measure the partial pressures of oxygen. Three optrodes were attached to the three calorimeter sample ampules through the lids, see Fig. 1. The fourth reference cell of the calorimeter contained only an empty ampule.

Ampule lids were modified by adding two holes to accept the optrode and a 30 cm long, 24-gauge metal needle, see Fig. 2. The needle was soldered into the smaller hole with Stay-Brite solder, and then the optrode was sealed into the larger hole with silicon rubber glue. The optrode and needle extended about 2 mm beyond the inside surface of the ampule lid. The needle tubing and optic fiber passed through loose fitting holes in the two aluminum shunts above the ampule and were sealed into the large access lid with foam rubber stoppers. The ampule lid was sealed to the ampule with a flat rubber gasket. All seals were checked by submerging the assembly under water and pressurizing the system with a syringe.

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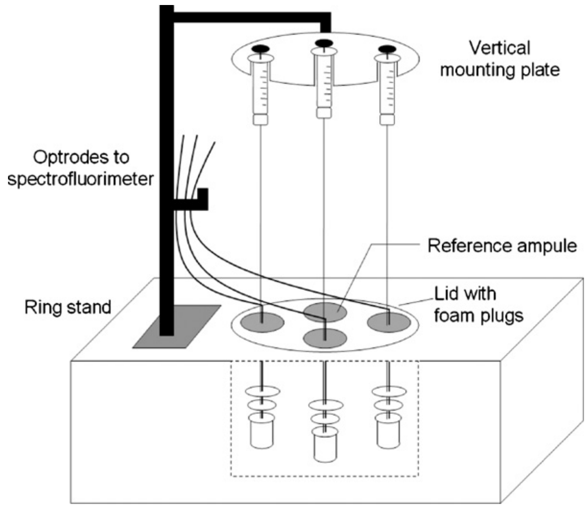

Fig. 1. Schematic of MC-DSC with three ampules and one common reference. Measurements can be made in triplicate with the system. A fourth cell (not pictured) is used as a reference in the calorimeter. A mounting plate holds the syringes in a vertical orientation and at the height to allow proper seating of the shunts and ampules in the calorimeter. A large lid with foam rubber plugs on top of the calorimeter isolates the calorimeter from air flow from the environment.

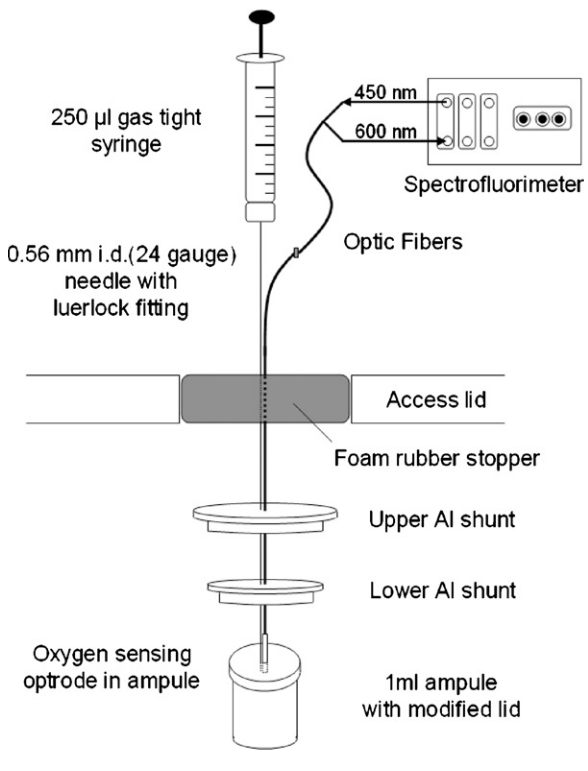

Fig. 2. Schematic of ampule with optrode and needle for calibration.

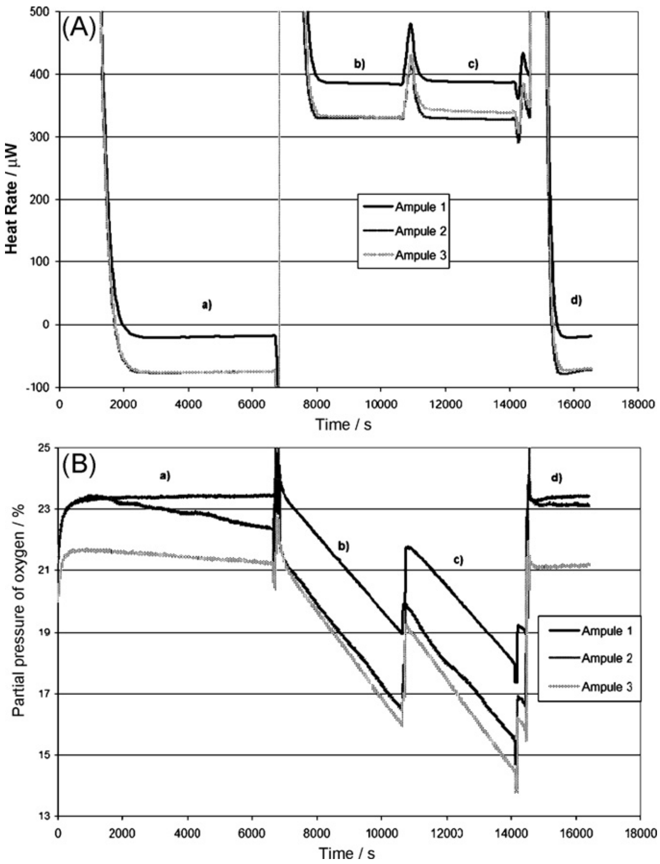

Fig. 3. Data from Zn–air batteries at 25 °C. The batteries were clamped in holders with a 5.6 kΩ resistor across the battery. (A) Heat rate: exothermic is upward, empty ampule baselines from 2000 to 7000 s and from 16,000 to 17,000 s; battery with holder and resistor inserted at 7000 s and removed at 15,000 s, optrode calibration at 11,000 and 15,000 s. (B) Partial pressure of O_2 from optrode measurements. The absolute value is only roughly calibrated.

Optrode measurements also showed no change in partial pressures of oxygen when the system was held at a constant pressure below and above atmospheric pressure, confirming that no oxygen was leaking in or out. With this system, the calorimeter required about 45 min to reach a steady state.

The FOXY oxygen sensor system consisted of the optrodes, bifuricated optic fibers, and three USB2000-FL Fluoresecence Spectrometers. A 450 nm light from the lamp is focused into one side of the bifuricated optic fiber. Partial pressures of oxygen are calculated from the light intensity measured at 600 nm from the other side of the fiber. The sensor does not introduce electrical fields into the ampule. Turning the excitation light on produces <2 µW increase in the baseline heat rate.

The optrode sensitivity is temperature dependent, the fluorescence signal decreases as temperature increases, but has a working

Table 1
Oxygen rates calculated from electrical current, calorimetric heat rates, and syringe calibration of optrodes. Steps (b) and (c) correspond to the time periods in Fig. 3.

	Battery voltage at 25 °C (V)	Resistance (Ω)	O_2 use rate from electric current (nmol s^{-1})	Heat rate (µW)	O_2 use rate from heat rate (nmol s^{-1})	O_2 rate from syringe calibration optrode (nmol s^{-1})
Ampule 1(b)	1.308	5608	0.6043	404	0.606	0.612
Ampule 1(c)				406	0.609	0.619
Ampule 2(b)	1.285	5637	0.5908	404	0.606	0.601
Ampule 2(c)				401	0.602	0.629
Ampule 3(b)	1.298	5631	0.5973	403	0.605	0.630
Ampule 3(c)				410	0.615	0.630

Table 2
Maple leaf bud respiration data. Segments (b) and (c) correspond to the data taken before and after injection of 150 μL of air to calibrate the optrode during the experiment.

	Wet weight (mg)	Dry weight (mg)	Heat rate (μW)	Oxygen rate (nmol O_2 s^{-1})	Heat rate/oxygen rate (kJ mol^{-1} O_2)
Ampule 1(b)	177	86.7	324	0.844	384
Ampule 1(c)			326	0.830	393
Ampule 2(b)	168	80.0	164	0.486	329
Ampule 2(c)			176	0.446	394
Ampule 3(b)	186	91.6	315	0.750	420
Ampule 3(c)			317	0.786	403

range from −60 to 80 °C. Therefore, only a rough calibration of the three optrodes was done before making measurements in the calorimeter ampules. This calibration was done by recording the fluorescence intensity with the sensors exposed to lab conditions (21% oxygen at 23 °C) outside the ampules. The ampules were then flushed with nitrogen gas as they were screwed onto the lids and fluorescence intensity recorded to obtain zero readings. The two values for each sensor were put into a linear Stern-Volmer equation in the OOISensors Software and used as an approximate guide to assess changes in oxygen partial pressure.

OOISensors application software was also used to log oxygen data. Partial pressures of oxygen were recorded at 6-s intervals. To accurately calibrate the optrodes in units of moles O_2 per change in signal, 150 μL of air at room temperature and pressure, and thus with a known number of moles of O_2 (1.10 μmol at 23.1 °C and 86 kPa by the ideal gas equation), was injected into the ampule with a 250 μL, gas-tight syringe (Hamilton Corporation; Reno, Nevada). The injection was done at the end of an experiment, see Fig. 3. To prevent diffusion of O_2 through the needle during a measurement of O_2 rate, the syringe was fully depressed and attached to the needle hub. To calibrate the optrode, the syringe was removed from the needle hub, filled to the 150 μL mark with air, reattached to the hub, and the calibration air injected.

The calibration was done this way for three reasons: the fluorescence signal sensitivity changes with temperature, the initial volume of air in the ampule with the sample is unknown, and since only the rate of oxygen consumption or production needs to be measured, it is unnecessary to determine the absolute partial pressure of oxygen. Both the oxygen and heat data were exported to Microsoft Excel for further analysis. The approximate partial pressures of oxygen in arbitrary units (percentage is convenient) were plotted against time and a polynomial fitted during times when heat rates from the samples were measured. A first-order equation can be used if heat and oxygen rates are constant. The slope of the fitting equation in arbitrary units s^{-1} was multiplied by the calibration value to obtain oxygen rates in mol of oxygen s^{-1}.

Duracell zinc–air batteries (DA-10 hearing aid battery [9]) were connected to spring clips cut from the ends of tubular fuse holders. One lead wire of a 5.6 kΩ resistor was soldered to one side of the holder and the other lead wire bent so as to make contact with the outside case of the battery. The other side of the clip was insulated with epoxy glue. The exact resistance of each holder without a battery and the voltage with a battery were measured with a Keithley model 195A digital volt-ohmmeter before insertion into the calorimeter. These values were used to calculate the current and thus predict the oxygen uptake rate by the battery. The stability of the battery system was determined to be better than 0.1% by recording the voltage of a parallel system for several hours outside the calorimeter. Before insertion into the calorimeter ampule, Scotch tape was placed around the holder to ensure no electrical contact with the hastelloy ampule. Heat rates and oxygen partial pressures were recorded at 25 °C. The zinc–air battery reaction is $2Zn + O_2 + 4OH^- + 2H_2O \rightarrow 2Zn(OH)_4{}^{2-}$, so the measured heat rates were also used to predict oxygen uptake rates. The package of resistor and battery produces −(666.5 ± 4) kJ/mol of O_2 at 25 °C [unpublished data, this laboratory].

Maple tree leaf buds (*Acer rubrum*) were gathered in early March by cutting small branches the day of the measurement and transporting these to the lab. In the lab, the buds were cut near the stem, weighed, placed in the ampule, and oxygen consumption rates and heat production rates measured at 25 °C.

3. Results

3.1. Zinc–air battery

The heat rates and partial pressures of oxygen measured in one experiment at 25 °C are plotted in Fig. 3. The heat production rates from the batteries were calculated by subtracting the baseline heat rates with empty ampules at the beginning and end of the experiment. The oxygen rate was calculated from a linear fit to the measured partial pressures of oxygen. Since the optrodes also exhibited some drift, a baseline oxygen rate was also determined from a fit of the partial pressure of oxygen data with the empty ampule at the beginning and end of the experiment. The oxygen rate was then calculated by multiplying the baseline corrected slope with the calibration from the injection of 150 μL of air. The results from the experiments shown in Fig. 3 are reported in Table 1. A calculation based on the Nernst equation shows the voltage in the battery did not significantly change with the change in partial pressure of oxygen in the ampule, i.e. E in ampule = E in air + 0.0591 $\log_{10}(p_{O_2}$ in ampule)/(p_{O_2} in air). Since the oxygen partial pressure only changes from 21% in air to about 15% at the end of the experiment, the voltage and current change by <0.2% during the experiment.

3.2. Maple buds

Oxygen and heat rates were recorded at 25 °C. Baseline corrections and calibration of the optrodes were performed in the same way as in the battery experiment. The measured heat rates were constant during the time of the oxygen measurements, so the optrode data were fit with a first-order equation to obtain oxygen rates. The results are given in Table 2.

4. Discussion

Oxygen consumption rates calculated from syringe calibration of the optrode are, respectively, 2 and 4% higher on average than oxygen rates calculated from the heat rate and calculated from the electrical current. Calorimeter baseline uncertainty of about ±5 μW and uncertainties in the enthalpy change for the reaction under the conditions in the battery probably account for the observed systematic difference between the calorimetric and amperometric results. The rates calculated from the syringe calibration of the optrode are probably high because of error in the measurement of the moles of oxygen injected during the calibration

. The needle tubing has a volume of 75 μL, and there may have been insufficient mixing of this volume of air with the air in the ampule. Reducing this error would require cycling the syringe after injection to ensure mixing, calibration of the volume of gas delivered by the syringe, replacing the Luer fittings on the current syringes with O-ring type seals, and using 150 μL syringes.

Growing plant tissues are expected to have a ratio of heat rate to oxygen rate equal to 455 ± 15 kJ/mol, the oxycaloric equivalent [10]. The values in Table 2 are on average 15% lower, part of which (2–4%) is accounted for by the systematic error in calibration of the optrode with the syringe. The remaining difference (13–11%) is apparently due to endothermic reactions in the tissue.

In summary, with proper attention to details of operation and construction, optrodes can be used in conjunction with calorimeters to make accurate, simultaneous measurements of oxygen rates and heat rates for metabolic and chemical reactions. Two cautions should be mentioned, the optrodes are rapidly damaged by volatile peroxides and optrodes have a limited use-life. Optrodes nearing this limit are unreliable as shown by variable calibration values.

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References

[1] J. Suurkuusk, I. Wadsö, Chem. Ser. 20 (1982) 155.
[2] E. Gnaiger, J. Exp. Zool. 228 (1983) 471.
[3] E. Gnaiger, in: E. Gnaiger, H. Forstner (Eds.), Polarographic Oxygen Sensors: Aquatic and Physiological Applications, Springer-Verlag, New York, 1983, pp. 134–166.
[4] P. Bäckman, I. Wadsö, J. Biochem. Biophys. Methods 23 (1991) 283–293.
[5] P. Johansson, I. Wadsö, Thermochim. Acta 342 (1999) 19–29.
[6] F. Ullrich, M. Winkelmann, R. Hűttl, G. Wolf, Anal. Bioanal. Chem. 383 (2005) 747–751.
[7] R.S. Criddle, A.J. Fontana, D.R. Rank, D. Paige, L.D. Hansen, R.W. Breidenbach, Anal. Biochem. 194 (1991) 413–417.
[8] A.J. Fontana, K.L. Hilt, D. Paige, L.D. Hansen, R.S. Criddle, Thermochim. Acta 258 (1995) 1–15.
[9] http://www.duracell.com/oem/Primary/Zinc/Zinc_Air_Tech_Bulletin.pdf (accessed September 7, 2008).
[10] L.D. Hansen, C. Macfarlane, N. McKinnon, B.N. Smith, R.S. Criddle, Thermochim. Acta 422 (2004) 55–61.