0273-2289/03/104/0001/$20.00

Continuous Oxygen Monitoring of Mammalian Cell Growth on Space Shuttle Mission STS-93 with a Novel Radioluminescent Oxygen Sensor

Julie S. Reece,[1] Michael J. Miller,[2] Mark A. Arnold,*[,1] Cris Waterhouse,[3] Ted Delaplaine,[3] Laura Cohn,[3] and Tom Cannon[3]

Departments of [1]Chemistry and [2]Physics and Astronomy, 230 IATL, The University of Iowa, Iowa City, IA 52242, E-mail: mark-arnold@uiowa.edu; and [3]Department of Resuscitative Medicine, Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD

Received April 1, 2002; Revised June 1, 2002; Accepted June 1, 2002

Abstract

A compact, flow-through oxygen sensor device based on luminescence quenching was used to monitor dissolved oxygen levels during mammalian cell growth on the STS-93 mission of the Columbia space shuttle. Excitation of an oxygen-sensitive ruthenium complex was provided by a radioluminescent light source (0.9 mm in diameter, 2.5 mm long), and the intensity of the resulting luminescence was measured by a simple photodiode detector. The use of radioluminescence for the excitation light source is a unique approach that provides many features important for long-term and remote monitoring applications. For the spaceflight experiment, human lung fibroblast cells (WI-38) were grown in hollow-fiber bioreactors. Oxygen concentration was measured in the flow path both before and after the bioreactor cartridge in order to gain information about the metabolism of the cells. The sensor was found to be nonperturbing to cell growth and withstood the challenging physical conditions of shuttle launch and landing while maintaining a stable calibration function. In addition, the sensor provided physically meaningful oxygen predictions.

Index Entries: Oxygen sensors; optical sensors; ruthenium fluorescence quenching ; radioluminescence; spaceflight monitoring; bioreactor monitoring.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

Reliable and robust sensing technologies are needed to monitor and control biologic experiments both in space and on Earth. Dissolved oxygen (DO) concentration is important because of its role in cellular physiology. The ability to monitor this analyte continuously during cell cultivation enables tight control and enhanced growth conditions. Despite this need, very few options are available that are amenable to the conditions encountered.

Optical oxygen detection schemes offer an alternative to the inherent disadvantages of the more commonly used electrochemical method. Although the electrochemical technique offers a fast, linear, and sensitive response, its key limitation is poor long-term stability. Response times are in the subminute regime *(1,2)*, and the range covered by these probes is approx 0.001–1000 ppm *(3)*. Signal drift and biofouling of the membrane limit its use in long-duration work *(3)*. In addition, the sensing reaction consumes oxygen, which can lead to a systematic measurement error when measuring low-volume or low-oxygen samples *(4)*.

By contrast, the optical technique does not consume oxygen and is quite sensitive, stable, and specific toward molecular oxygen. In this method, the luminescence of an indicator complex is efficiently quenched in the presense of oxygen. Ruthenium complexes are generally preferred for their long luminescent lifetimes, excellent quenching and quantum efficiencies, strong visible absorptions, and large Stokes' shifts. The indicator complex used in this work, tris (4,7-diphenyl-1,10-phenanthroline) ruthenium (II) chloride ($Ru[dpp]_3Cl_2$), is well known for its excellent sensitivity to oxygen *(5–8)*. Much research has been done in this field in the last couple of decades, and there are commercial products now reaching the marketplace based on this technology *(9–11)*.

The device reported herein is unique in its choice of excitation light—radioluminescence. Radioluminescent (RL) light is produced when ionizing radiation from a radioisotope excites a phosphor to produce light of a desired color. The RL source used in the reported sensor is a miniature glass capsule internally coated with an inorganic phosphor and filled with gaseous tritium. The beta particles produced by the tritium decay are too weak to penetrate through the glass so there is no external leakage of radioactivity. Although the dim nature of RL sources leads to low signals, its extremely low noise levels make favorable signal-to-noise ratios attainable *(12)*. Photobleaching of the indicator complex is negligible owing to the low radiant powers, further improving the useful life of the sensor and its calibration function. In addition, their small size and zero power requirement make these sources ideal for a spaceflight application in which both space and power are at a premium *(13)*.

This article details the deployment of a compact and robust oxygen sensor device *(12,14)* on a spaceflight application to monitor cellular metabolism. The biology component of our experiment was designed to

probe the changes in physiology and gene expression on exposure of WI-38 cells to the microgravity environment. Extended spaceflight travel is associated with atrophy in heart, muscle, and bone, but on an accelerated time scale when compared to normal aging processes on Earth. Thus, these microgravity experiments provide a novel experimental model system for the study of aging *(15,16)*.

The RL oxygen sensor was integrated into the NASA/WRAIR Cell Culture Module (CCM) aboard the space shuttle. The CCM is a self-contained, automated hardware unit housed in a standard space shuttle middeck locker and contains four hermetically sealed bioreactor and fluidpath assemblies with accompanying controllers and data loggers. STS-93 was a test flight for the CCM-C, an experimental modification to the CCM that contains a cooling chamber for storage in place of one bioreactor/fluidpath assembly. The sensor was developed, tested, calibrated, and sterilized at the University of Iowa and also recalibrated in Iowa postflight. The Iowa calibration data were then used to process data collected at the Kennedy Space Center in Florida and aboard the Columbia space shuttle. The device was nonperturbing to the cells in the bioreactor system and was able to physically withstand the rigorous conditions encountered. Calibration stability of the sensor over the course of the lengthy and demanding experiment is discussed herein as well as the oxygen levels predicted by the device.

Materials and Methods

Toluene and methylene chloride were from Fisher (Pittsburgh, PA). Titanium dioxide (99.9%+, 5-μm maximum particle size) was obtained from Aldrich (Milwaukee, WI). Monarch 120 carbon black was a product of Cabot (Boston, MA). Acetic acid–releasing, one-component silicone prepolymer was a product of Dow Corning (Midland, MI) sold as a common household clear silicone sealant. $Ru(dpp)_3Cl_2$ was from laboratory stock. Digital gas mass flow controllers (Cole-Parmer, Vernon Hills, IL) were used to generate gas streams of known oxygen concentration using nitrogen as a makeup gas. Metricide-activated dialdehyde solution (MX-1400) obtained from Metrex (Parker, CO) was used as the sterilizing agent for the optical sensor according to the manufacturer's instructions.

The membrane cocktail was composed of 0.025 g of TiO_2 powder, 0.15 g of silicone adhesive, and 0.3 mL of a 1 m*M* solution of $Ru(dpp)_3Cl_2$ in methylene chloride. The cocktail was prepared in a small glass vial and thoroughly stirred with a glass rod to ensure homogeneity. The cocktail was applied to the interior of a glass capillary tube (3 mm od, 2 mm id) with a peristaltic pump at 2 mL/min. Once the casting solution had run through, the tube was removed from the cocktail solution and the residual solution was pumped out of the capillary. The tube was then flushed with air for at least 1 min to solidify the membrane and was stored in a vertical position for full curing in air. After the initial ruthenium-silicone layer was fully

cured, a coating of black silicone was applied for optical isolation. The casting solution for this layer was composed of 0.02 g of carbon black, 0.24 g of silicone adhesive, and 0.5 mL of toluene and was applied in a similar fashion. Once both sensing layers were fully cured, the tube was washed with plenty of water.

Blue, tritium-powered RL light sources (0.9 mm in diameter, 2.5 mm long, 3 mCi tritium activity) were obtained from mb-microtec ag (Niederwangen/Bern, Switzerland). A Hamamatsu (Bridgewater, NJ) photodiode (G1736) was used as the sensor detector element. A red, long-pass Schott glass filter (OG590) was obtained from Optical (Houston, TX) and was fastened onto the photodiode face using optically clear Epotek 301 epoxy from Epoxy Technology (Billerica, MA).

A schematic of one optical channel is shown in Fig. 1. The chemistry-coated capillary tube is located above the photodiode detector-filter assembly. The chemistry tube serves as the flow path, and in Fig. 1, the flow is perpendicular to the page. The RL source is glued into a small aluminum block and positioned near the chemistry tube at about 90° relative to the detector. The RL source is external to the sample flow path.

In Fig. 1, light from the RL source propagates through the glass matrix of the capillary tube and into the ruthenium-silicone layer. The ruthenium-silicone layer is separated from the sample by a thin optical isolation layer. This isolation layer restricts the detected light to the ruthenium layer and prevents the adverse impact of absorbing sample molecules. A fraction of the light emitted from the ruthenium layer is detected by the nearby photodiode.

Figure 2A shows the entire sensing device. This device has external dimensions of $4 \times 3 \times 1.5$ in. It contains eight individual oxygen-sensing flow channels and all the necessary detector and amplification circuitry. Figure 2B is a view of one side of the box without the enclosing faceplate. Four individual oxygen-sensing units are shown. For each unit, a detector is positioned at the back wall of the optical cavity, and the glass capillary, internally coated with the chemistry, is joined to metal flow connectors with plastic tubing to comprise the flow path. The aluminum block for housing the RL sources is also visible. An aluminum faceplate (not shown) fits over all units to minimize ambient light effects.

A schematic representation of the experimental design used for the space shuttle mission is presented in Fig. 3. Sterile medium is pumped from the medium reservoir through an oxygenator membrane and one channel of the RL oxygen sensor. The medium then travels through the hollow-fiber bioreactor inoculated with human lung fibroblast (WI-38) cells and through a second oxygen channel. This configuration results in two oxygen measurements per bioreactor. The difference between the pre- and postreactor measurements corresponds to oxygen consumed by the cells and can be related to cell metabolism rates.

For the space shuttle work, three sensing devices were constructed: one as a flight unit, one as a ground control unit, and a third as a backup.

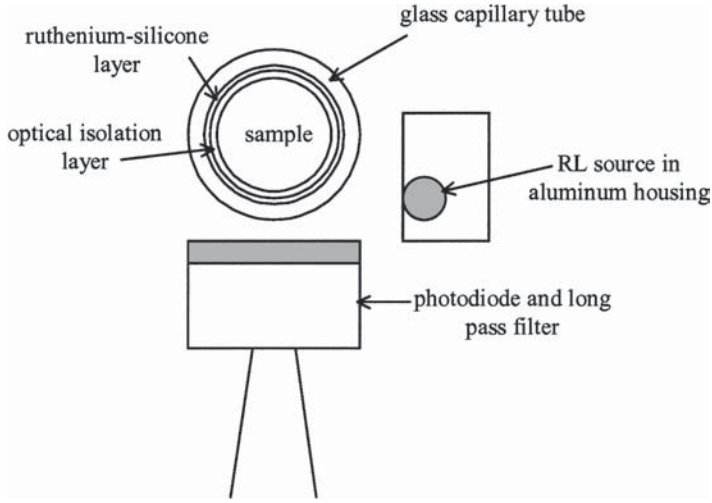

Fig. 1. Capillary optical sensor layout with self-powered RL source.

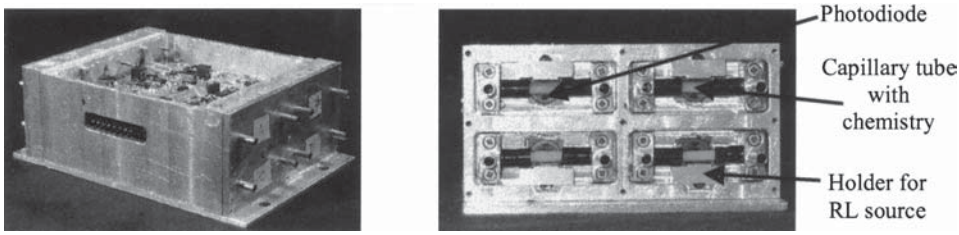

Fig. 2. Oxygen-sensing device. **(A)** Entire box housing containing eight unique measurement channels; **(B)** closeup of four optical cavities.

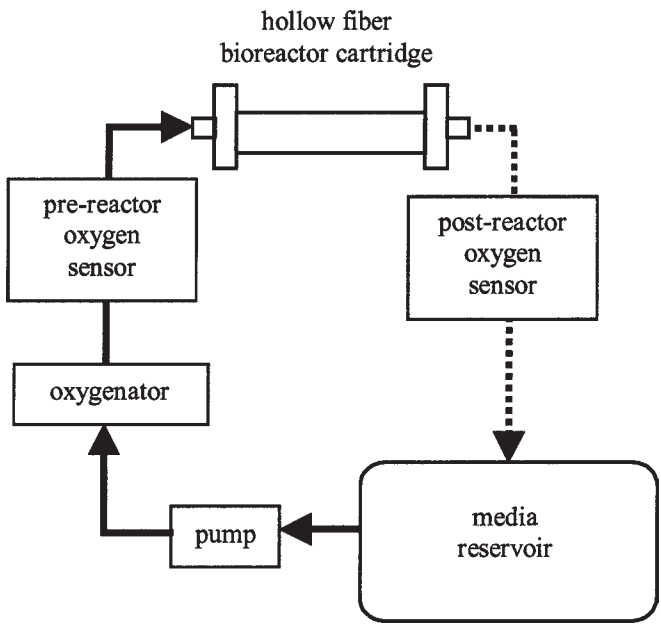

Fig. 3. Solution flow path for monitoring DO in cell culture module. Solid lines represent flow of prereactor medium. Dashed lines represent flow of postreactor medium. Oxygen measurements are made before and after each bioreactor cartridge.

The devices were tested, calibrated, and sterilized at the University of Iowa and shipped to the Kennedy Space Center in Florida. Once in Florida, the boxes were powered up for preliminary testing and flow-path priming. Data were collected nearly every day for all channels. The sensors were finally integrated into the NASA/WRAIR CCM (CCM-C) for the flight. The STS-93 mission was initially set to begin on July 20, 1999. There were two subsequent launch delays and the actual launch occurred on July 23, 1999. The flight time was approx 107 h, with subsequent landing on July 27, 1999. After landing, the sensing devices were shipped back to the University of Iowa for postflight recalibration and processing.

Results and Discussion

In this work, the RL-based oxygen sensor was used to continuously monitor oxygen levels during mammalian cell growth on the STS-93 mission of the Columbia space shuttle. The RL sensor flow path was integrated into the CCM-C for concurrent spaceflight and ground-based experiments. Unique challenges were involved in designing and building an analytical instrument for spaceflight. The arduous conditions of launch and landing could make an accurate measurement difficult. Calibration stability from Iowa to the experiment in Florida and aboard the shuttle was also a challenge. Finally, practical considerations regarding power consumption, physical size, and ease of operation and integration became important.

The sensor device described herein represents a novel application of radioluminescence for excitation of a luminescent indicator complex. Radioluminescence has seen relatively little use in the field of analytical chemistry primarily because of low radiant powers, which necessitate high amplification of the resulting signal. RL sources have many practical advantages that merit their use in this type of application. Long-term calibration stability is very important for an application such as the space shuttle, in which frequent recalibration is not feasible. The output of the RL sources exhibits low noise levels and negligible drift with time. In addition, photobleaching of the indicator dye is minimized, thereby enhancing calibration stability. These two features combine to produce a sensor with a long calibration lifetime. In addition, their small size and zero power requirement were important practical considerations for remote applications.

The sensor device was characterized thoroughly in the laboratory prior to the flight and exhibited favorable response times, limit of detection, and calibration stability. The aqueous response time (98%) of the device for the transition from nitrogen- to air-saturated water is about 1 min, while that for the reverse is 3 min. These response times are sufficient for the intended application, since oxygen changes in bioprocesses occur on the timescale of minutes or hours. The limit of detection of the sensor is 1.35 ± 0.89 torr and the standard error of prediction is 3.0 torr when measuring aqueous samples ranging from 0 to 159.5 torr over a time period of 11 d. The calibration demands of the shuttle work were especially challenging because the sensors were calibrated in Iowa

and these calibration factors were subsequently used to analyze data collected both in Florida and during the space shuttle mission.

The physical layout of the device was designed as a flow-through sensor. The oxygen-sensitive indicator complex is immobilized in a gas-permeable silicone polymer and cast onto the interior of a glass capillary tube. A black optical isolation layer is then coated on top of the chemistry layer. In this design, the optical properties of the sample, such as color or turbidity, will have no effect on the measured signal. This feature is important because the cellular growth medium used for the mission contained phenol red as an *in situ* colorimetric pH indicator.

The space available for the RL sensor in the CCM-C was limited, which necessitated a compact design. The complete sensing module contained eight individual oxygen-sensing channels as well as the required electronic circuitry to measure the current output from each photodiode detector. Raw detector currents were amplified by 12 orders of magnitude, and a low-pass filter was used to reduce noise levels. Each of the eight optical channels contained its own detector, long-pass glass filter, ruthenium-containing sensing tube, and blue RL source.

There were two primary qualitative objectives for sensor performance during the space shuttle mission. First, it was desired that the sensor not interfere with the cellular growth within the hollow-fiber cartridge. This issue is important because some ruthenium complexes can be toxic to cells *(17)*. We found no inhibition of cell growth in either ground-based or flight units, which indicates that immobilizing the indicator dye in a silicone polymer prevents leaching and permits normal cell growth. In a related experiment, the Alamar blue toxicity test with WI-38 cells was performed with the ruthenium membranes, and no growth inhibition was observed (A. S. Jeevarajan, personal communication). We have also used this device with a variety of cell types and have observed no sensor-related toxicity effects. Second, we wanted to demonstrate a robust sensor design that would withstand the challenging physical conditions encountered during both launch and landing. The analytical measurement, luminescence intensity, is quite sensitive to the alignment of the various optical components *(7)*. When the device was returned to Iowa after the flight, a physical inspection confirmed that all optical compartments were intact with no obvious changes. In addition, the device performed well throughout all stages of the experiment, including the launch and landing time periods.

Demonstrating the quantitative capabilities of the device was also important. In previous laboratory testing, long-term calibration stability was established. Drift of the sensor output is another key parameter. In the shuttle experiment, the drift of the prereactor channel was approx 0.26 mV/d compared to an operating voltage range of about 400 mV over 0–100% air saturation. This degree of drift translates into an error of about ±0.5 torr at air saturation. Because of the nonlinear nature of the calibration function, the error induced by this drift would be lower at oxygen concentrations below air saturation.

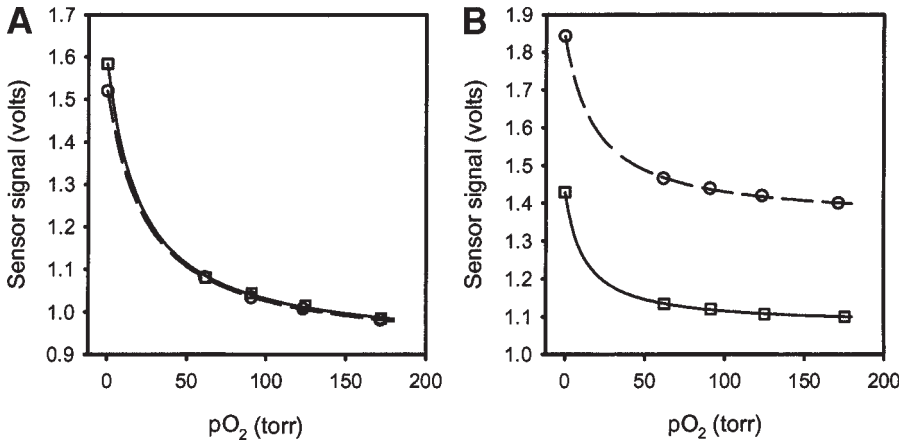

Fig. 4. Comparison of pre- and postflight calibration data. (—□—) Preflight data collected in Iowa; (– – ○ – –) postflight data also collected in Iowa. **(A)** Example of sensor channel that exhibited excellent stability and **(B)** example of sensor channel that exhibited voltage shift during experiment.

A comparison of pre- and postflight calibration data showed significant voltage shifts in some of the sensor channels. The plots in Fig. 4 are representative of the best and worst cases. In Fig. 4A, there is very little difference between the pre- and postflight calibrations, thus demonstrating the robustness of the module's design. However, some channels, as illustrated in Fig. 4B, demonstrated significant voltage shifts that would make accurate oxygen predictions impossible. Of the channels exhibiting such shifts, there was no specific trend in terms of the direction or magnitude of the shift. However, although the absolute voltage was offset, the basic sensitivity and range of the calibration were maintained in each case.

The cause of the voltage shifts appears to be electronic, rather than optical or chemical, in nature. On careful analysis, it was determined that each channel shift was an isolated, onetime event that was evident after initial power-up at the Kennedy Space Center following shipment from Iowa. Indeed, sensor voltages collected in Florida for several days prior to the flight, during the flight, and at Iowa after the flight concur, thus demonstrating the stability and robustness of the device. It would have been preferable to use preflight calibration data for analysis. However, owing to the voltage shifts and the lack of full-scale calibration data in Florida, the postflight Iowa calibrations were used for data processing.

Figure 5A presents data collected from one sensor pair during the space shuttle mission. The arrows represent shuttle launch and landing. At these time points, the sensor is functioning normally and there is no shift in the voltage output or deterioration in data quality. The CCM-C temperature is also shown in Fig. 5A, and there is an obvious correlation between the observed sensor voltage and the ambient temperature of the self-contained CCM-C. The CCM-C temperature fluctuations were in part

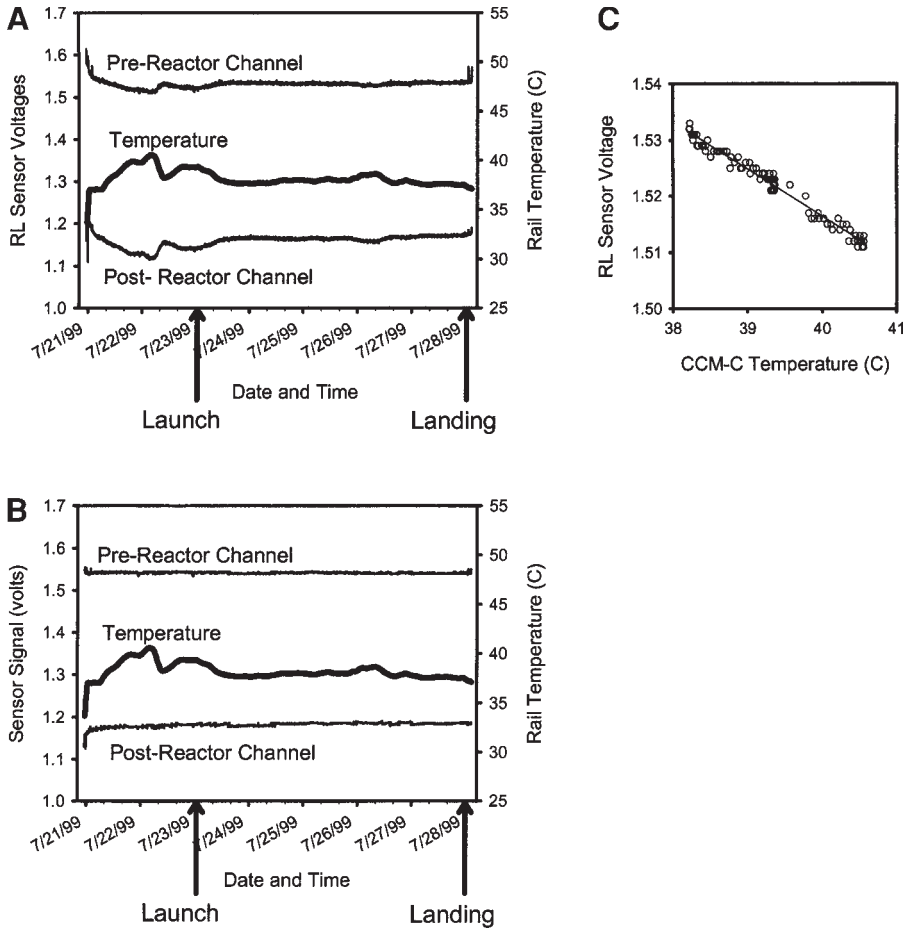

Fig. 5. Data collection from space shuttle experiment from one bioreactor pathway. Arrows indicate the times of launch and landing of the space shuttle. **(A)** Raw data output for pre- and postreactor oxygen channels and measured temperature; **(C)** sensor voltage collected for 24-h period prior to launch plotted vs measured temperature; **(B)** same data as in **(A)** plotted after temperature normalization.

owing to the experimental status of the cooling chamber and accompanying temperature control modifications. The temperature sensitivity exhibited by the sensor channels is actually greater in magnitude than the oxygen sensitivity. Thus, oxygen predictions derived from the raw data would be erroneously influenced by the temperature. It was therefore necessary to remove this confounding temperature information from the sensor outputs.

Figure 5C demonstrates the strong correlation between output voltage and temperature for one sensor channel during the period prior to launch. The slope of the corresponding line provides a measure of the temperature coefficient, k_{temp}. A different temperature coefficient was computed for each channel. Although there are a variety of chemical contribu-

tions to the temperature effect *(13,18–20)*, in our device, the electronic contribution dominates.

The temperature coefficient was then used to remove the temperature information from the sensor signals via the following equation:

$$V_N = V + [k_{temp} \times (T_{calib} - T)] \quad (1)$$

in which V and V_N are the observed and normalized voltages, respectively; T and T_{calib} are the observed and calibration temperatures, respectively; and k_{temp} is the temperature coefficient. The result of this operation is shown in Fig. 5B. The temperature-induced voltage fluctuations are efficiently removed with this calculation, which allows for accurate oxygen predictions from V_N.

Despite limited information against which to compare, there is strong evidence that the sensors did in fact perform reliably. Two of the three sensor boxes were used; one flew on the space shuttle and the second was mounted in an identical, ground-control experiment. Of the 16 analytical channels between these two boxes, 15 predicted physically reasonable oxygen concentrations ($0 < pO_2 < 159.5$ torr). An analysis of the sensor pairs from different bioreactor flow paths is also valuable. Typically, the prereactor channels predicted oxygen concentrations ranging from 60 to 150 torr, while postreactor channels were generally at 0–50 torr. Cellular consumption of oxygen occurs in the hollow-fiber bioreactor cartridge. Thus, the postreactor oxygen level should be lower than the prereactor level. Of the eight oxygen sensor pairs tested, six demonstrated this trend.

Conclusion

A novel optical oxygen-sensing device utilizing an RL light source was used for bioreactor monitoring aboard the Columbia space shuttle. The compact sensing device, capable of eight simultaneous measurements, was conveniently integrated into the NASA/WRAIR CCM-C. The sensor demonstrated robust calibrations despite the challenging conditions, and physically meaningful oxygen predictions were obtained. The key performance advantage of this sensor is its long-term calibration stability. This advantage results primarily from using a novel radioluminescent light for excitation of the oxygen-sensitive indicator complex. In addition, the sources were favorable from a practical standpoint because of their small size and zero power requirement. The flow-through design integrates well with different bioreactor systems, and we have also developed a probe-style device for additional flexibility in implementation. The concept of this type of source is general in nature and could be readily extended to other fluorescent indicator-analyte systems by using the appropriate RL emission wavelength. A colorimetric approach could also be envisioned, although the lower sensitivity of this technique would pose a challenge given the low radiant powers of these sources. This type of technology shows great promise for applications in addition to the space shuttle such as on the International Space Station or for extended environmental field studies.

Acknowledgments

We wish to acknowledge Jennifer Winkenwerder at the University of Iowa for assistance with instrument manufacture and testing. We also wish to thank Dr. Eugenia Wang and McGill University, Montreal, Canada, for collaboration with their WI-38 experiment; Cheryl Lacsamana from WRAIR for her assistance with integrating the sensor into the CCM; and the NASA/WRAIR/Space Test Program for the flight opportunity. Financial support for the sensor was from the Microgravity Science and Applications Division of NASA (grant no. NAG9-824 and NAG8-1592).

References

1. Orna, M. V. (1989), in *Electrochemistry, Past and Present*, vol. 390, Stock, J. T. and Orna, M. V., eds., American Chemical Society, Washington, DC, pp. 196–210.
2. Harris, D. C. (1995), *Quantitative Chemical Analysis*, W. H. Freeman, New York.
3. Turner, A. P. F. and White, S. F. (1999), in *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*, vol. 4, Flickinger, M. C. and Drew, S. W., eds., Wiley, New York, pp. 2056–2070.
4. Chuang, H. and Arnold, M. A. (1999), *Pure Appl. Chem.* **71,** 803–810.
5. Demas, J. N. and DeGraff, B. A. (1991), *Anal. Chem.* **63,** 829A–837A.
6. Kneas, K. A., Demas, J. N., DeGraff, B. A., and Periasamy, A. (2000), *Microsc. Microanal.* **6,** 551–561.
7. Demas, J. N., DeGraff, B. A., and Coleman, P. B. (1999), *Anal. Chem.* **71,** 793A–800A.
8. Carraway, E. R., Demas, J. N., DeGraff, B. A., and Bacon, J. R. (1991), *Anal. Chem.* **63,** 337–342.
9. Oxygen Sensor for Respiration Monitoring, Ocean Optics, www.oceanoptics.com/products/respirationmonitor.asp. Accessed May 2002.
10. Photosense LLC/Products and Technology, www.photosense.com/products.html. Accessed May 2002.
11. Griffiths, J. R. and Robinson, S. P. (1999), *Br. J. Radiol.* **72,** 627–630.
12. Chuang, H. and Arnold, M. A. (1997), *Anal. Chem.* **69,** 1899–1903.
13. Demas, J. N. and DeGraff, B. A. (1992), in *Chemical, Biochemical, and Environmental Fiber Sensors IV*, vol. 1796, Lieberman, R. A., ed., International Society for Optical Engineering, Bellingham, WA, pp. 71–75.
14. Chuang, H. and Arnold, M. A. (1998), US patent 5708957.
15. Wang, E. (1999), *FASEB J.* **13,** S167–S174.
16. Semov, A., Semova, N., Lacelle, C., Marcotte, R., Petroulakis, E., Proestou, G., and Wang, E. (2002), *FASEB J.* **16,** 899–901.
17. White, D. O., Harris, A. W., Cheyne, I. M., Shew, M., Shulman, A., and Bradley, T. R. (1969), *Aust. J. Exp. Biol. Med. Sci.* **47,** 81–89.
18. Van Houten, J. and Watts, R. J. (1976), *J. Am. Chem. Soc.* **98,** 4853–4858.
19. Demas, J. N. and DeGraff, B. A. (1993), *Sens. Actuators B* **11,** 35–41.
20. Draxler, S. (1999), *J. Phys. Chem. A* **103,** 4719–4722.