

## An Optical Oxygen Sensor for Long-term Continuous Monitoring of Dissolved Oxygen in Perfused Bioreactors

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For long-term growth of mammalian cells in perfused bioreactors, it is essential to monitor the concentration of dissolved oxygen (DO) present in the culture medium to quantitate and control level of DO. Continuous measurement of the amount of DO in the cell culture medium in-line under sterile conditions in NASA's perfused bioreactor requires that the oxygen sensor provide increased sensitivity and be sterilizable and non-toxic. Additionally, long-term cell culture experiments require that the calibration be maintained several weeks or months. Although there are a number of sensors for dissolved oxygen on the market and under development elsewhere, very few meet these stringent conditions.

An optical oxygen sensor (HOXY) based on dynamic fluorescent quenching and a pulsed blue LED light source was developed in our laboratory to address these requirements. Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride is employed as the fluorescent dye indicator. The sensing element consists of a glass capillary (OD 4.0 mm; ID 2.0 mm) coated internally with a thin layer of the fluorescent dye in silicone matrix and overlaid with a black shielding layer. Irradiation of the sensing element with blue light (blue LED with emission maximum at 475 nm) generates a red fluorescence centered at 626 nm. The fluorescence intensity is correlated to the concentration of DO present in the culture medium, following the modified non-linear Stern-Volmer equation. By using a pulsed irradiating light source, the problem of dye-bleaching, which is often encountered in long-term continuous measurements of this type, is minimized. To date we achieved sensor resolution of 0.3 mmHg at 50 mmHg pO<sub>2</sub>, and 0.6 mmHg at 100 mmHg pO<sub>2</sub>, with a response time of about one minute. Calibration was accomplished in sterile phosphate-buffered saline with a blood-gas analyzer (BGA) measurement as reference. Stand-alone software was also developed to control the sensor and bioreactor as well as to acquire data.

Two HOXY sensors with a single calibration were employed to continuously monitor the DO in GTSF-2 medium during a Baby Hamster Kidney (BHK-21) cell culture in a Rotating Wall Perfused Vessel (RWPV) bioreactor for 90 days. HOXY sensors were located at the inlet to and outlet from the bioreactor. One of the sensors was placed between an oxygenator and the inlet to the bioreactor. The dissolved oxygen concentrations determined by both sensors were compared with those measured regularly with the BGA reference. The cell culture was maintained for 110 days. Sensor output was found to correlate well with the BGA data throughout the experiment, where the DO of the medium ranged between 25 and 50 mmHg at the bioreactor outlet and 90-130 mmHg at the bioreactor inlet. Measuring DO with the HOXY sensors versus the BGA reference indicated bias values of -2 mmHg and -15 mmHg, and precision values of  $\pm 3$  mmHg and  $\pm 16$  mmHg at the bioreactor inlet and outlet, respectively. (Supported by NASA: NAS9-97114)