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INSTRUCTIONS FOR USE

723 MICRO OXYGEN ELECTRODE

NOT INTENDED FOR USE ON HUMANS

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1. GENERAL INFORMATION

A. DESCRIPTION

Diamond General's 723 Microelectrodes are intended for use in biological and physiological in vivo or in vitro systems. It is a monopolar electrode, and thus, requires the use of an external reference electrode. (see ACCESSORIES, Section VI.)

B. SYSTEM THEORY

1. THEORY OF POLAROGRAPHIC OXYGEN MEASUREMENTS

When two electrodes are polarized with a potential of slightly less than -1.0 volt in an electrolytic solution containing dissolved oxygen, current will flow as a result of the reduction of oxygen at the cathodic (negatively polarized) surface. This reaction at the cathode is expressed as:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$$

At the other electrode, described as the reference, oxidation takes place. For an Ag/AgCl reference, the reaction is:

$$4Ag + 4Cl^- \rightarrow 4AgCl + 4e^-$$

The voltage-current relationship for a polarographic oxygen electrode is represented by the characteristic curve (Figure 1). In the region below approximately -0.5 volt, there is a reasonably linear voltage-current relationship. As the polarization voltage is increased beyond -0.5 volt, the current will tend to reach a plateau in which changes in voltage have little effect on current. In this plateau region, the current is limited by the rate at which oxygen can diffuse to the cathode. As the voltage is increased above -1.0 volt, the current will again increase with voltage, due to the reduction of other elements in addition to oxygen.

The electrode is normally operated with the polarization voltage set to the midpoint of the plateau region, in which case the current is diffusion limited. In a diffusion-limited condition, virtually all of the oxygen molecules which reach the cathode are immediately reduced, resulting in a zero oxygen concentration at the cathode surface, and a current which is limited by the rate at which oxygen can diffuse to this zero concentration region. The diffusion rate is a function of the oxygen diffusion coefficient of the membrane and media surrounding the cathode and the dissolved oxygen concentration which, in turn, is proportional to the oxygen partial pressure and temperature.

The result is that, for a constant temperature, current flow through the electrode will be directly proportional to the partial pressure (PO2) of oxygen.

A plot of the relation between current and PO2 (at a fixed polarization voltage) is called the standard curve (Figure 2). For most electrodes, the curve is linear. It should be noted that the curve does not intersect the origin, but rather, indicates a small current at zero PO2. This current is called the residual, or "dark current," and results from factors such as electrical leakage through insulating materials in the system and reduction of oxygen which was absorbed into the electrode materials.

Figure 1

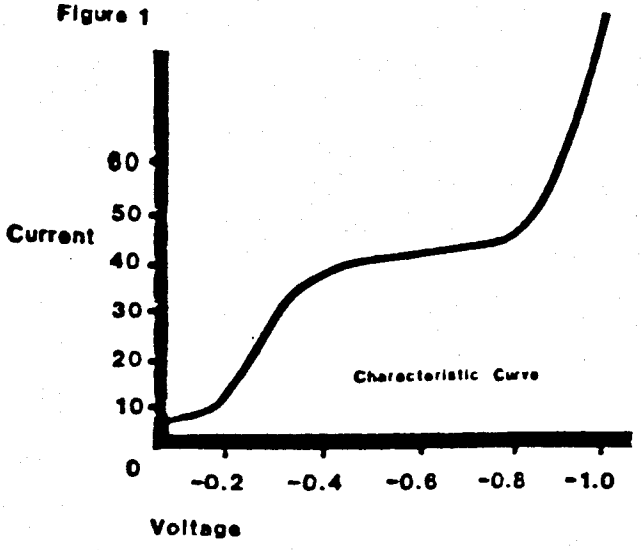
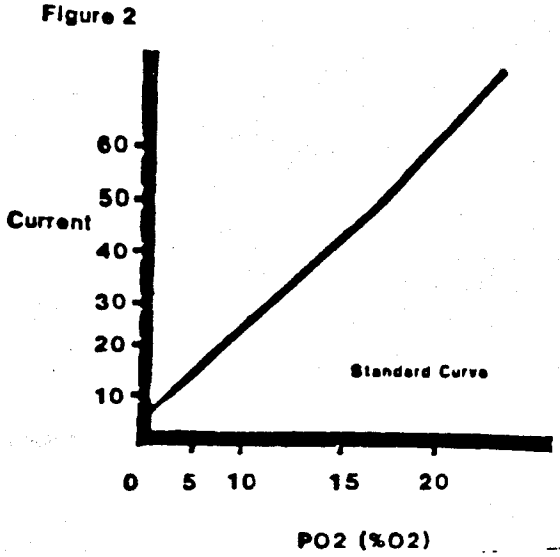


Figure 2



2. PRACTICAL CHARACTERISTICS OF OXYGEN ELECTRODES

When using miniature and micro-sized oxygen electrodes, the electrode characteristics are sometimes less than ideal. The smaller the electrode, the more difficult it is to maintain identical characteristics from one electrode to the next. Also, the wide variety of applications in which these electrodes are used makes it difficult to optimize the system for a particular application. However, with a proper understanding of the electrode characteristics, accurate oxygen measurements can be obtained.

The shape of the characteristic curve (Figure 1) can vary considerably between electrodes and also can change with shelf life and use. The plateau is frequently not flat, but might have a small positive or negative slope. also, it might cover a span of 0.1 to 0.4 volt in width, with midpoint occurring anywhere between 0.5 and 0.95 volts.

If we define the plateau as the region of the characteristic curve which has the minimum slope and operate the electrode at a voltage occurring near the midpoint of this region, the

standard curve will be approximately linear (Figure 2). Occasionally, the characteristic curve might vary considerably from the classic and have a poorly defined plateau, but the linearity of the standard curve is not simultaneously degraded.

II. ELECTRODE SPECIFICATIONS

Length	7.0 cm
Tip Diameter	2.5 microns ± 0.5 micron
Shaft Diameter	1.0 mm
Typical Current (Ambient PO2)	70 picoamps
Time Constant (0-90%)	<4 seconds
Membrane Thickness	7-10 microns
Temperature Coefficient	+5% per degrees C

III. PRECAUTIONS

A. THIS ELECTRODE IS NOT INTENDED FOR USE ON HUMANS.

B. These electrodes are EXTREMELY fragile. See Sec. IV A. HANDLING, before use.

C. Inspect the electrodes for breakage visually before breaking seal on box. This is best achieved by viewing under a 10X objective through the clear plastic box.

D. To extend life of electrode, polarize no longer than 2 hours before calibration.

IV. PROCEDURE

A. HANDLING

Extreme care is necessary in handling the electrodes, as mechanical contact with materials can easily break the tip. If the electrode is to be inserted into tissue or any material other than a liquid, it must be done using a micromanipulator, keeping all forces parallel to the axis of the electrode. When bubbling gases through test solutions to calibrate the electrode, make certain that the electrode tip is not directly in the flow of bubbles, as this can create breakage.

Inspect the electrodes for breakage visually before breaking seal on box. This is best achieved by viewing under a 10X objective through the clear plastic box.

B. OPERATION

Note: An external reference electrode must be used.

1. THE SELECTION OF POLARIZATION VOLTAGE AND PRODUCTION OF THE CHARACTERISTIC CURVE.

Set up the amplifier following the appropriate instructions. Most oxygen electrodes function well when polarized with a potential of -.75 volts; therefore, it is not really necessary for the user to produce a characteristic curve. Simply stabilize the electrode at the operating voltage (-.75 v) and proceed to Section IV B, 2. If you wish to determine the optimum polarization voltage, you may produce your own characteristic curve by following this procedure:

- If the electrode has not yet been in use, you must allow the electrode to stabilize by soaking the tip for only 2 hours in physiological saline or other calibrating media with reference electrode at a potential of -0.75 V applied.

- To extend life of electrode polarize no longer than 2 hours before calibration.

- Set the amplifier to indicate current in picoameres. (If using DG's 1201 Chemical Microsensor, set the COARSE GAIN to 10(-10), the COARSE ZERO to 10(-12), both the FINE GAIN and FINE ZERO full counterclockwise).

- Equilibrate a beaker of physiological saline (80 ml if using DG's Calibration Cell) with room air (approximately 21% oxygen).

- Set the polarization voltage on the amplifier to an initial value of -1.0 volt.

- Allow several minutes for the reading to stabilbize. A strip chart recorder (1.0 volt full scale sensitivity) will aid in observing stabilization.

- Record the current reading and decrease the polarization voltage to -.95 volts.

- Repeat the above procedure at 0.05 volt intervals allowing the electrode to stabilize at each voltage change.

- Plot the characteristic curve (current vs. voltage) and note the plateau defined as the region of minimum slope. The voltage at the midpoint of the plateau should be chosen as the operating voltage for the electrode.

2. STABILIZATION

Allow the electrode to stabilize at the operating voltage (normally -.75 V) and at the temperature at which measurements are going to be made for at the most 2 hours. A strip chart recorder will aid in observing stabilization.

3. CALIBRATION

Note: A reference electrode must be used in the calibration media as well as the measurement media.

Calibration must be carried out at the same temperature at which the measurement site will be. Since oxygen partial pressure and current have a linear relationship, a 2-point calibration will suffice for most applications.

The simplest type of calibration involves using 21% O2 (ambient room air) and 0% O2 (100% nitrogen) to produce 2 calibration points. If measurements are limited to a very small range and high accuracy is required, we recommend that an oxygen-nitrogen gas mixture, which has an oxygen concentration similar to that of the substance being tested, be used in place of the air. 100% nitrogen should always be used to determine the zero point, thereby allowing compensation for the "dark current."

First, bubble the higher level O2 gas through the solution containing the electrode. Allow 10 to 15 minutes for the solution to equilibrate with the gas. If using the Chemical Microsensor, dial the **FINE GAIN** until the displayed PO2 matches that of precalibrated gas. (The **COARSE GAIN** should be preset to 10(-10)). Set the **COARSE ZERO** two orders of magnitude above that of **COARSE GAIN**. Example: If **COARSE GAIN** is set to 10(-10), **COARSE ZERO** should be set to 10(-12).

Next, bubble 100% nitrogen until the solution is saturated. As previously mentioned, there will be small residual or "dark current" displayed. This small offset (normally less than 10% of the direct current reading at 21% O2) must be subtracted electronically at the amplifier. On the Chemical Microsensor, the **FINE ZERO** control is used. Set the **FINE ZERO** until a reading of 00.0 is displayed on the amplifier. Repeat this procedure (with both gases) readjusting the **FINE GAIN** as necessary to complete an accurate calibration. The electrode is now ready to be removed from the calibration media and inserted into the experimental media (see PRECAUTIONS, III B). A reference electrode must also be used in the experimental media. The reference electrode does not need to be adjacent to the 723 Microelectrode as long as there is electrical conduction or continuity between the two electrodes.

Note: If the electrode has been subjected to a temperature change while transferring from the calibrating media to the experimental media, it may take several minutes to restabilize in the experimental media. Also, polarization voltage is temporarily disconnected when electrode is removed from solution. For these reasons, it is important to transfer the electrode as quickly as possible.

4. DRIFT

Regardless of the calibration methods chosen, it is important to realize that some electrode will drift slightly, even after their initial stabilization. For this reason, we recommend that when high accuracy is required, a calibration procedure be utilized both before and after the experiment. If the experiment is over several hours in length and the experiment design will allow for it, additional calibration checks during the experiment would be advisable.

5. NOISE

The noise level of the electrode is sufficiently low that it will not interfere with accurate PO2 readings. If excessive noise is observed, it is probably due to external sources. The system is susceptible to external interference due to the extremely low currents and high impedances involved. Good grounding and shielding practices should be observed, and operation in a cage is sometimes advisable. Also, movement of people in the immediate vicinity can cause current fluctuations due to the resulting stray capacitance variations.

V. ELECTRODE MAINTENANCE

A. STORAGE

When not in use, the electrodes should be stored dry in a dust free container, such as a desiccator. Be sure to store them so that the tips are not toughing any surface, since this would probably result in breakage.

B. CLEANING

Due to the nature of the butylacetate membrane, it is impossible to use the 723 Microelectrodes indefinitely without some build-up or contamination in the tip. With proper care, the electrode can be used many times over.

After use, submerse the tip of the electrode for 15-30 minutes in deionized H2O to remove salts from the membrane.

If using the electrode in a protein rich solution, soaking the electrode in a protease solution (an enzyme that breaks up proteins) can be helpful in cleaning the tip. Follow by soaking in deionized H2O.

VI. RECOMMENDED ACCESSORIES

Chemical Microsensor (Product # 1201)

Calibration Cell (Product # 1251)

Miniature Microelectrode Holder (Product # 1108)

Reference Electrode (Product # 334)

Electrode Cable (Product # 1150)

Chemical Microsensor II (Product # 1231)