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CHEMICAL MICROSENSOR

MODEL 1201

OPERATING AND MAINTENANCE MANUAL

TG PT. NO. 9062



TRANSIDYNE GENERAL CORPORATION

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## I. DESCRIPTION OF INSTRUMENT

### A. GENERAL DESCRIPTION

Transidyne General's Chemical Microsensor is a research instrument for the amperometric measurement of the partial pressure of dissolved oxygen, or  $PO_2$ . When combined with the Calibration Cell (Model 1251) and any of the polarographic electrodes available at Transidyne General, it provides a complete system for in vivo or in vitro  $PO_2$  measurements.

The digital meter displays  $PO_2$  in either millimeters of mercury (mm Hg) or as a percentage of total pressure, alternately, it can provide a direct measurement of polarographic current. Analog outputs are provided for recording the  $PO_2$  (or polarographic current) and polarization voltage on a strip-chart recorder or other device.

### B. SPECIFICATIONS

INPUT CURRENT RANGE: The Coarse Gain settings are  $10^{-6}$  to  $10^{-10}$  amp full scale in decade steps, with 0.1 picoamp the least significant digit at the  $10^{-10}$  gain setting. The Fine gain control is continuously variable from the Coarse Gain setting to approximately x 11 additional gain.

ZERO CURRENT RANGE: Adjustable from  $10^{-6}$  to  $10^{-12}$  amp.

ELECTRODE POLARIZATION RANGE: The full-scale range is  $\pm 1$  V continuously variable with a 10-turn control knob and polarity switch. With the Meter Selector switch in the Electrode Polarization position, the voltage is readable to 1% full-scale accuracy.

ABSOLUTE ACCURACY:  $\pm 2\%$

LINEARITY:  $\pm 1\%$

DRIFT: Less than 0.5% per 24 hr after 0.5 hr warm-up period.

ANALOG OUTPUT:  $\pm 1.999$  V with a 200-ohm source resistance.  
Maximum output current is 5 ma.

ELECTRODE POLARIZATION OUTPUT:  $\pm 1.0$  V.

DIGITAL DISPLAY: The 3-1/2-digit display indicates either electrode polarization voltage or  $O_2$  level as selected by the Meter Selector switch. (The meter range =  $\pm 1.999$  V.) The Output Selector switch allows  $O_2$  to be displayed as percent,  $O_2$  mm Hg, or mm Hg expanded scale. Maximum meter ranges are 100%, 1999 mm Hg, and 1999.9 mm Hg expanded scale.

POWER REQUIREMENTS: 105-125 VAC, 50-60 Hz, 7.5 watts.

210-250 VAC, 50-60 Hz, 7.5 watts.

Line voltage set with back panel switch

FUSE: 0.25 amp, Slow Blow, MDL 1/4.

DIMENSIONS: 12.5 x 7.5 x 6.75 in.

WEIGHT: 7.5 lb.

### C. RECOMMENDED ACCESSORIES

#### (1) Calibration Cell

The Calibration Cell provides (a) a means of dissolving gas mixtures and then exposing these mixtures to the electrode, and (b) a temperature control maintaining an accuracy of  $\pm 0.02^\circ\text{C}$  over a range of  $20^\circ$  to  $65^\circ\text{C}$ . Both of these features are essential for oxygen electrode calibration.

#### (2) Oxygen/Hydrogen Electrodes

Transidyne General offers 3 types of oxygen/hydrogen electrodes: (a) a microelectrode, (b) a needle style electrode, and (c) a Clark Style electrode. For detailed information, refer to the instructions and specifications sheet enclosed with your electrode.

#### (3) Reference Electrode

This sintered silver/silver chloride non polarizable electrode is for use with our microelectrode (model 723) or our needle electrode (model 760), not our Clark electrodes (model 730 or 731).

(4) Miniature Microelectrode Holder (Model 1108).

The input connector is housed in a 0.25 x 4 inch stainless steel holder which can be clamped to a micromanipulator or the Calibration Cell. It has a 40 inch shielded cable with a BNC connector which attaches directly to the Chemical Microsensor. The holder is for use with Transidyne's microelectrode (model 723) and reference electrode (model 334).

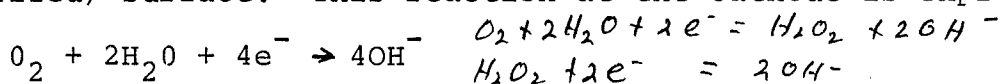
(5) Oxygen Uptake Equipment

Transidyne General offers a complete set of oxygen uptake equipment. For detailed information, refer to the instructions enclosed with your oxygen uptake equipment, or contact our customer service department.

## II. POLAROGRAPHIC OXYGEN MEASUREMENTS

### A. THEORY OF POLAROGRAPHIC OXYGEN MEASUREMENTS

When two electrodes are polarized with a potential of slightly less than negative one volt in a solution containing electrolytes and 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:

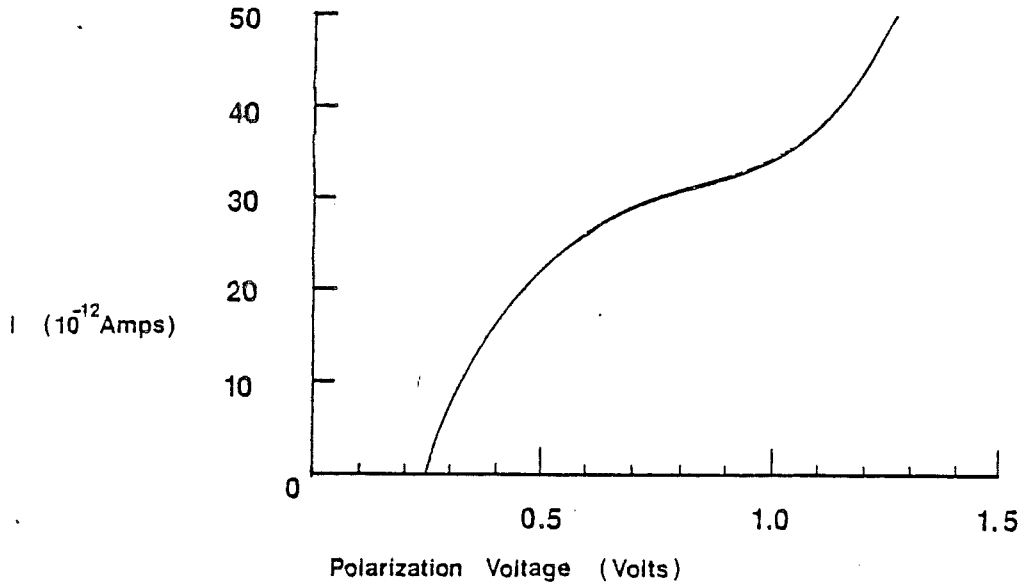


At the other electrode, described as the reference, oxidation takes place. For a Ag/AgCl reference, the reaction is:



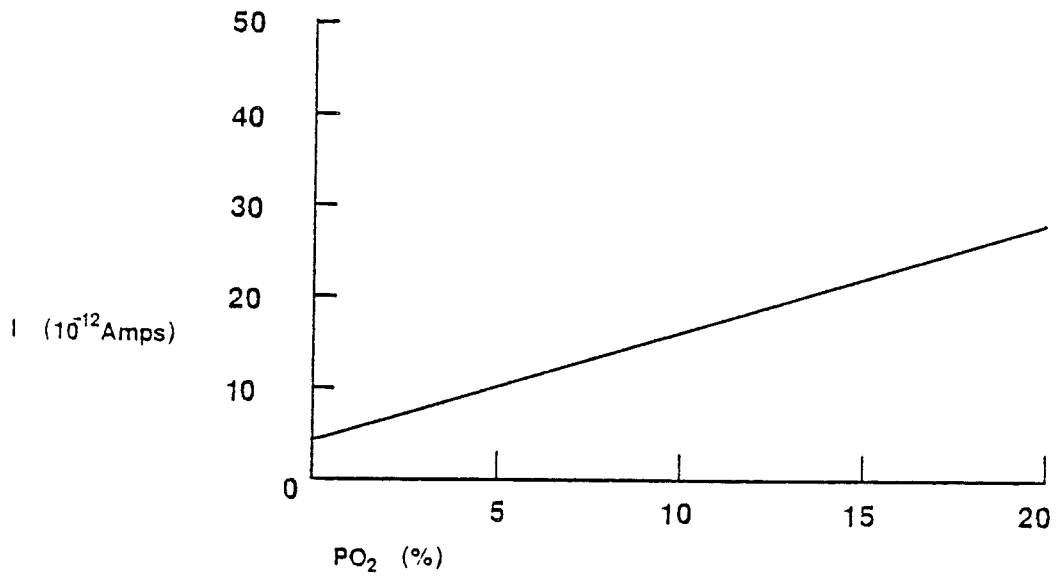
The voltage-current relationship for a polarographic oxygen electrode, is represented by the characteristic curve (Fig. 2.1). In the region below approximately -0.5 volt, there is a reasonably linear voltage-current relationship. As the polarization voltage is increased beyond -0.5 volt, the current 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.



Characteristic Curve

FIGURE 2.1



Standard Curve

FIGURE 2.2



The result is that, for a constant temperature, current flow through the electrode will be directly proportional to the partial pressure ( $PO_2$ ) of oxygen.\*

A plot of the relation between current and  $PO_2$  (for a fixed polarization voltage) is called the standard curve, as shown in Figure 2.2. 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  $PO_2$ . 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.

#### B. 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 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 a midpoint occurring anywhere between 0.5 and 0.95 volt.

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\*The term "tension" is frequently used in medical and biological literature in place of "partial pressure." The two expressions are equivalent, though "partial pressure" is often considered preferable.

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. 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.

All oxygen electrodes tend to drift, particularly upon initial operation. Drift is particularly apparent in solutions containing proteins, and even more so in the presence of hemoglobin. It occurs as a result of large molecules attaching themselves to the cathode, changing its surface area and operating characteristics. A membrane will protect the cathodic surface from direct contact with interfering molecules and thus substantially reduce the drift, though not necessarily eliminate it.

Drift is minimized by "aging" the electrode while polarized in the type of solution in which  $PO_2$  measurements are to be made. After several hours to several days of operation, the electrode will usually stabilize and thereafter drift only a few percent.

Temperature is an additional consideration. Since diffusion rates vary with temperature, so will the calibration of the oxygen electrode. Therefore, it is necessary to calibrate the electrode at the same temperature as that of the experimental subject or material.

For details concerning your electrode, see the manual enclosed with the electrode.

### III. OPERATION

#### A. CONTROL AND CONNECTOR FUNCTIONS

##### 1. FRONT PANEL CONTROLS (See Figure 3.1)

a. POWER This AC power switch is illuminated when the instrument is on.

b. METER This control determines the function of the DIGITAL DISPLAY. When set to OUTPUT, the display responds to polarographic current and can be calibrated either in terms of partial pressure or absolute current. When set to ELECTRODE POLARIZATION, the electrode polarization voltage is displayed. This control has no effect on the output signals obtained from the OUTPUT connector.

c. HOLD This contact switch allows the displayed value to be temporarily held as an aid in recording rapidly changing PO<sub>2</sub> levels. This switch does not affect the signal from the OUTPUT connector.

d. OUTPUT This control sets the scale factor on the digital display for the desired units. The % position displays the PO<sub>2</sub> as a percentage of atmospheric pressure and is also used for absolute current measurements. The mm Hg position displays the PO<sub>2</sub> in pressure units of millimeters of mercury; values will be 7.6 times those displayed with the control set to %, since 100% PO<sub>2</sub> corresponds to 760 mm Hg, or atmospheric pressure. mm Hg EXP is an expanded scale in which the gain is increased by a factor of 10 over the mm Hg scale or 76 over the % scale, thus increasing the resolution. Any time these scale factors are changed, the decimal point on the display always appears at the correct position for the units indicated.

e. COARSE GAIN Sets the range of current gain. The settings indicate the magnitude of current which produces a display of 100.0 when the OUTPUT control is set to % and

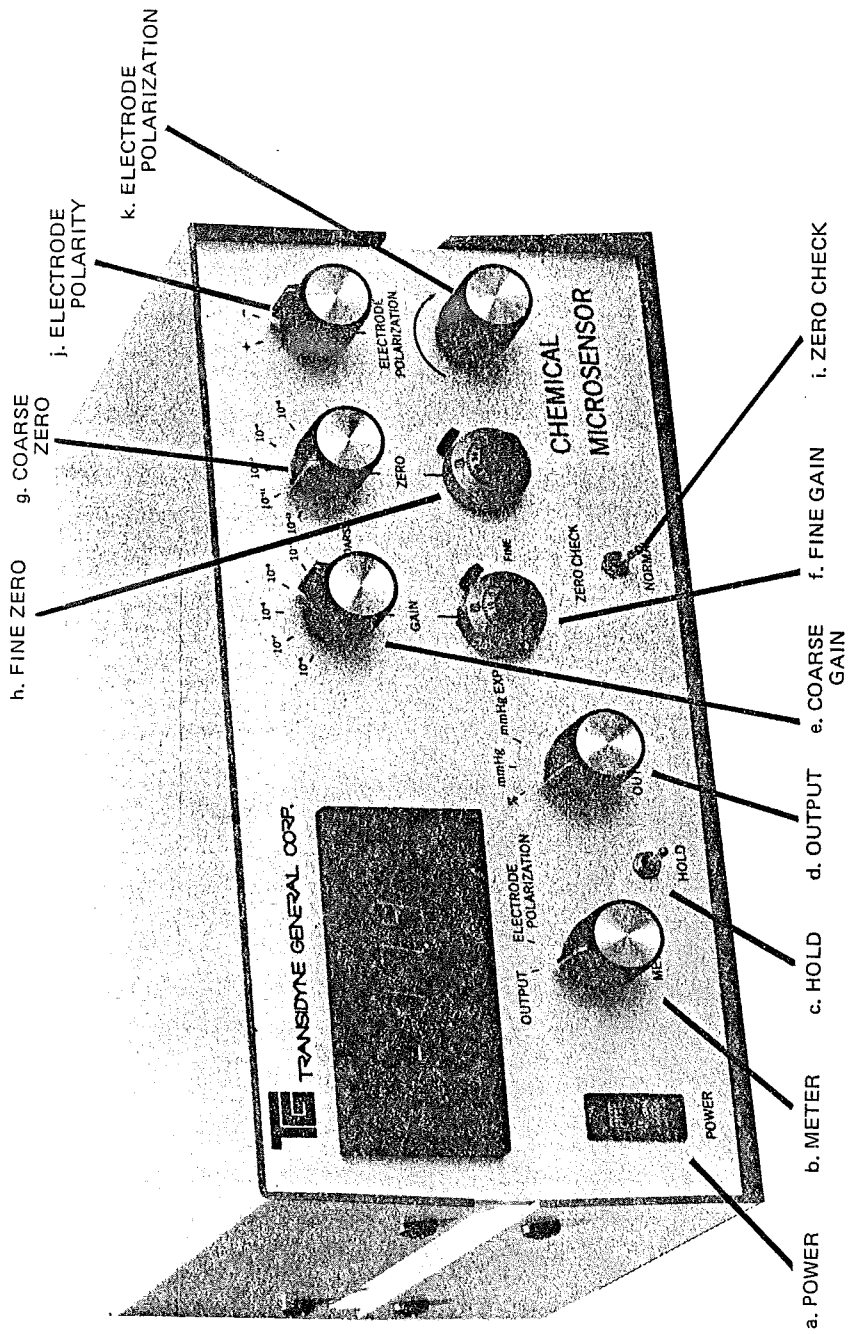


Figure 3.1 The Chemical MicroSensor -- Front Panel

FINE GAIN is at its full counterclockwise position. For example, if the COARSE GAIN is set to  $10^{-10}$ , with OUTPUT at  $\frac{8}{10}$  and FINE GAIN counterclockwise, the display will be in units of picoamperes ( $10^{-12}$  ampere), with 0.1 picoampere the least significant digit.

f. FINE GAIN Varies the gain, as set by the COURSE GAIN control, by a factor of about 11.

g. COARSE ZERO Sets the range of zeroing current, which is used to compensate for the residual current typically seen with oxygen electrodes in a zero  $PO_2$  environment. The settings indicate the scale of the midrange decade of zeroing current. (See FINE ZERO control).

h. FINE ZERO Varies the zeroing current over about a three decade range, with the lowest setting (at the full counterclockwise position) one order of magnitude below that set on the COARSE ZERO control.

i. ZERO CHECK/NORMAL When this switch is set to "Zero Check", the electrode is disconnected from the current amplifier and the polarization voltage removed. The zeroing current, however, is still connected to the amplifier and can be measured at this time. To check the magnitude of the zeroing current relative to the polarographic current, flip to "ZERO CHECK" position. If the electrode has been calibrated, the display will indicate a negative value proportional to the residual (or zero) current. Prior to calibration, i.e., with the fine zero full counterclockwise, the display will read zero + 1.5.

j. ELECTRODE POLARITY Sets the polarity of the potential applied to the electrode. The polarity of the current amplifier is internally adjusted so that polarographic current is always displayed as positive. For oxygen electrodes, this

control is always set to negative (-); there are, however, other types of polarographic measurements, such as dissolved hydrogen determination, which require a positive electrode potential.

k. ELECTRODE POLARIZATION This control varies the polarization potential applied to the electrode over a range of 0.0 to 1.0 volt.

## 2. BACK PANEL TERMINALS AND CONTROLS (See Figure 3.2)

a. INPUT Connector to which the polarographic electrode is attached. The inner conductor is for the active electrode, while the outer conductor is used for a shield. This shield connection can also be used for the reference input when the active and reference electrodes are in the same assembly, such as a Clark electrode.

b. REFERENCE Reference electrode input. The inner and outer conductors are in parallel and connected to chassis ground.

c. OUTPUT Provides an analog output signal which is proportional to polarographic current. A 1.0-volt output corresponds to a digital panel meter reading of 1000 (disregarding position of decimal point). 2.0 volts is the maximum output. This output is unaffected by the setting of the METER switch.

d. ELECTRODE POLARIZATION The electrode polarization output provides a signal which is equal to the polarization voltage applied to the electrode. Maximum signal is 1.0 volt.

e. LINE VOLTAGE SWITCH Sets the instrument for either 115 or 120 VAC operation. WARNING: THE SWITCH MUST BE SET TO THE PROPER LINE VOLTAGE BEFORE THE INSTRUMENT IS PLUGGED IN. OTHERWISE, THE INSTRUMENT MAY BE SEVERELY DAMAGED.

f. FUSE Fuse holder for a 0.25-amp, slow blow fuse, type MDL 1/4.

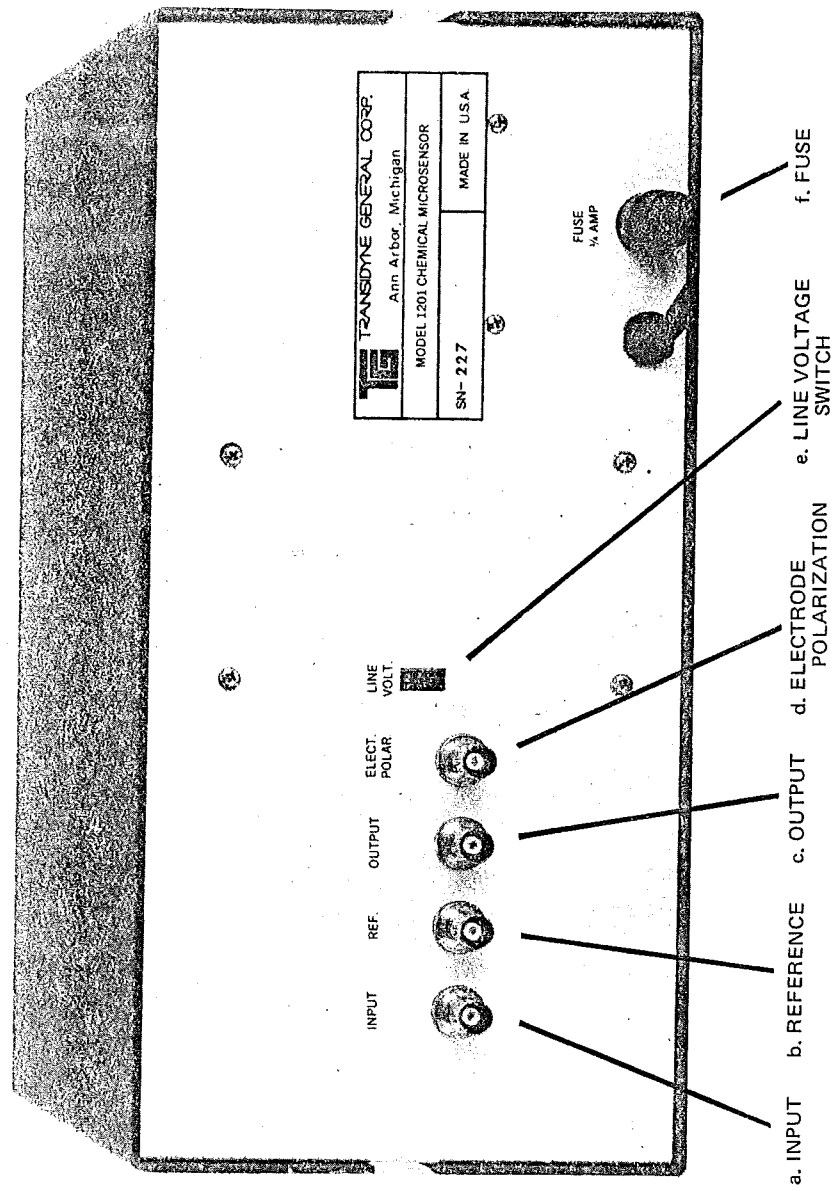


Figure 3.2 The Chemical MicroSensor -- Rear Panel

## B. INSTRUMENT PREPARATION

### 1. POWER REQUIREMENTS AND WARM-UP

The instrument is designed to operate on either 105-125 VAC or 210-250 VAC, 50-60 Hz. The line voltage is selected by the LINE VOLTAGE SWITCH on the back of the instrument. MAKE SURE THAT THE LINE VOLTAGE SWITCH IS SET TO THE APPROPRIATE VOLTAGE BEFORE PLUGGING IN THE INSTRUMENT.

The instrument should be plugged into a grounded receptacle and turned on at least one-half hour prior to use.

### 2. CONNECTIONS TO EXTERNAL EQUIPMENT

#### a. Sensing Electrodes

(1) Our microelectrode (Cat. # 723) is connected to the input connector via our Miniature Micro electrode Holder (Cat. # 1108), otherwise, a high quality coaxial cable should be used.

(2) The needle electrode (Cat. #760) is provided with a 30" shielded cable. The connection is also made via the input connector.

(3) Our Clark electrode (Cat. # 730) is connected to the input via our Clark electrode cable (Cat. #77104). Here, the "built in" reference electrode is connected to the shield of the coaxial cable, thereby grounding it.

b. The reference electrode is connected to the REFERENCE input. Since the outer and inner conductors of this input are both connected to ground, the type of cable used is not critical. Our miniature electrode holder (Cat. #1108) can be used for the connection.

When the system is calibrated by means of the Calibration Cell (Model 1251), the reference electrode built into the Cell



is connected to the REFERENCE input. When using a Clark electrode, do not connect this reference electrode to the amplifier. Leaving the built in reference electrode submersed in the same solution with the Clark electrode will not affect the reading, providing the reference electrode is NOT connected to the amplifier. Before the measurements are taken, the reference electrode to be used during the experiment is connected to the instrument. If the reference electrode used during the experiment is a type other than Ag/AgCl (that used in the Calibration Cell), then it should be placed in the Cell and used during the calibration procedure. The same type of reference electrode must be used for both calibration and measurement.

c. Use of the OUTPUT connector is optional. For most applications, however, it is desirable to connect this output to a strip chart recorder. The recorder is extremely useful for observing the stabilization during the calibration procedure (see Section 3.3), as well as for monitoring changes in  $PO_2$  during the experiment. A 1.0-volt, full scale span will cover the full range of the Microsensor, while a 250 or 500 mV span is useful for the lower concentrations normally encountered.

d. THE ELECTRODE POLARIZATION output provides an alternative means by which the polarization voltage can be measured however, since the potential can be measured on the digital display and is not changed during a measurement, this output is seldom needed.

One application in which some users might find this signal helpful is the plotting of polarographic curves (see Section 3). Normally, the voltage and current are read from the digital display and plotted manually. However, if an X-Y plotter is available, the ELECTRODE POLARIZATION can be connected to the X-axis and the OUTPUT (which represents polarographic current) to the Y-axis. The plotter sensitivity should be set for a 1.0-volt full span on both axes. The plot should be made as a series of points rather than a continuous line, since the pen

will have to be lifted each time the polarization voltage is changed in order to allow the electrode to stabilize at the new voltage.

### 3. INITIAL CONTROL SETTINGS

This action will prepare your instrument for calibration. The typical initial control settings should be as follows (See Section III-A for a functional description of the controls).

- a. POWER - ON Let instrument warm up at least  $\frac{1}{2}$  hour prior to use.
- b. ELECTRODE POLARITY - Negative for oxygen measurements. Positive for hydrogen measurements.
- c. METER - Electrode Polarization to display the voltage. The voltage is adjusted with the Electrode Polarization Control. Proper voltage varies slightly with the type of electrode used, so refer to the instruction sheet enclosed with your electrode. Switch to OUTPUT to display current or oxygen concentration. Switching the meter switch from ELECTRODE POLARIZATION to OUTPUT does not affect the polarizing voltage to the electrode.
- d. OUTPUT - Set to units desired, either % Oxygen or mm Hg.
- e. ZERO CHECK - Since some electrodes can be damaged by applying excessive voltage, it is advisable to keep this switch at the ZERO CHECK position prior to setting the polarization voltage. When the switch is in this position, it disconnects the polarizing voltage to the electrode. When the electrode is in position, and the proper voltage adjusted, flip to NORMAL.

- f. FINE ZERO - Full counterclockwise (minimum current).
- g. COARSE ZERO - Full counterclockwise (minimum current).
- h. COARSE GAIN - Full counterclockwise, unless proper gain range is known through experience with the electrodes.
- i. FINE GAIN - Full counterclockwise.

## C. ELECTRODE CALIBRATION PROCEDURE

### 1. HANDLING

Extreme care must be used for handling our glass microelectrodes. The needle and Clark electrode are considerably more durable and can be handled without danger of breakage.

Refer to your electrode instruction sheet for instruction regarding the handling and care of your electrode.

### 2. PREPARATION OF CALIBRATION CELL (Cat. #1251) & ELECTRODE FOR DETAILED SPECIFICATIONS SEE CALIBRATION CELL MANUAL.

Fill the tank to about 2 cm from the tip with distilled H<sub>2</sub>O and the beaker with about 80ml of physiological saline (0.9% NaCl). Position the gas dispersion tube so that the bubbles are directed against the side of the beaker rather than into the center where they might contact the electrode.

Adjust the temperature controller to the temperature at which oxygen measurements will be made.

Assuming current and percent oxygen are linear only two known gas concentrations are required. Zero O<sub>2</sub> (100% N<sub>2</sub>), and Ambient (21% O<sub>2</sub>) are sufficient.

The gas supplies should be connected and the Pressure adjusted so that a moderate stream of bubbles is released. The calibration cells "built in" reference electrode is used with the monopolar electrodes (Microelectrode #723, and Needle electrode #760). It should be connected to the reference input on the calibration cell and used through the entire calibration.

When using the Clark style electrode (#730 or 731), do not connect the reference electrode to the Chemical Microsensor, since the Clark electrode contains its own "built in" reference electrode. If you have chosen to use other equipment for

calibration, keep in mind the factors that are vital for accurate calibration:

1. You must calibrate the electrode at the same (+ 0.2°C) temperature at which you are going to be making measurements.

2. You must expose the electrode to at least 2 pre-calibrated gas concentrations.

### 3. SELECTION OF POLARIZATION VOLTAGE

Most oxygen electrodes will function well when polarized with a potential of -0.75 volt. However, we recommend that for optimum performance the user produce a characteristic curve and thus choose the most appropriate polarization voltage.

To produce a characteristic curve, the Chemical Micro-sensor must be set to display in units of current. The OUTPUT switch is set to  $\frac{1}{2}$ , the COARSE ZERO TO  $10^{-12}$ , and both the FINE GAIN and FINE ZERO to their full counterclockwise positions. The COARSE GAIN control settings are labeled to indicate the magnitude of full scale current (a meter reading of 100.0), as explained in section III, A; therefore, multiply by  $10^{-2}$  to obtain the magnitude of the units displayed. (For example, when the COARSE GAIN is set to  $10^{-8}$ , the actual units being displayed have a magnitude of  $10^{-10}$  amp.) Set the control initially to the  $10^{-6}$  range.

The beaker in the Calibration Cell should be filled with about 80 ml of 0.9% NaCl, which has been allowed to equilibrate with the gases in room air (21% oxygen). The temperature should be set to that at which the electrode will be used.

Carefully lower the electrode into the solution and set the polarization voltage to an initial value of -1.0 volt. The current through the electrode will take at least several minutes

to stabilize. A strip chart recorder (with a 1.0 volt full-scale sensitivity) connected to the OUTPUT of Model 1201 will aid in observing the electrode stabilization. When the electrode has stabilized at -1.0 volt, record the current and decrease the polarization voltage to -0.9 volt. Repeat the procedure at 0.1 - volt intervals, allowing the electrode to stabilize at each level. At some value below -0.5 volt, the current may reverse; this reversal is normal, and the procedure should be discontinued at this point.

After plotting the characteristic curve, observe 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. As the electrode ages, the shape of the characteristic curve will vary; however, the operating voltage chosen initially will usually remain in the plateau region.

#### 4. TYPICAL CALIBRATION PROCEDURE

##### A. STABILIZATION

The electrode must be stabilized prior to calibration by polarizing it at its operating voltage for at least several hours, preferably overnight, while immersed in saline. With some electrodes, better results are obtained if a solution similar to the one in which the measurements are to be made is used for the stabilization process.

The stabilization can be conveniently monitored by observing the electrode current on a strip chart recorder connected to the Chemical Microsensor OUTPUT. Because polarographic current is highly temperature sensitive, the temperature must be well regulated in order to avoid an apparent drift.

## B. CALIBRATION PROCEDURE

Set the controls on the Chemical Microsensor as follows:

Zero, Coarse & Fine	-	Full Counterclockwise
Gain, Coarse & Fine	-	Full Counterclockwise
Electrode Polarization	-	Appropriate voltage (See Section )
Meter	-	Output
Output	-	Units desired

The electrode should be stabilized as in Section 3 C. Leaving the electrode in the calibration cell, bubble room air into the calibrating solution for at least 5 minutes.

Adjust the coarse and fine gain to produce a meter reading of 21% or 159 mm Hg ( $PO_2$  of atmospheric oxygen).

Since some electrodes are motion sensitive, the bubbling must be stopped before a calibration point can be set.

Bubble 100% Nitrogen into the calibrating solution. When the reading has stabilized at it's near zero value (3-5 minutes), adjust the coarse and fine zero controls to subtract out this "residual" current, thus producing a reading of zero on the meter.

Adjustment of the zero controls will slightly offset the initial 21% calibration point. Bubble air through the chamber again and adjust the 21% value, using the fine gain. Follow this by nitrogen. After 1 or 2 iterations between air and nitrogen, the instrument will be calibrated.

## C. ABSOLUTE CURRENT MEASUREMENTS

The Chemical Microsensor can be used to measure in units of current rather than  $PO_2$ . This is particularly useful when working with a standard curve as described in sections 2 A and III C 3.

For most applications, no calibration is needed. The OUTPUT control is set to "%" and the FINE GAIN, FINE ZERO and COARSE ZERO controls are all set to their full counterclockwise positions. The COARSE GAIN is then set to the full scale current range desired. Since a full scale reading is "100.0," the actual units of the current being displayed will be two orders of magnitude less than the full scale current, or  $10^{-2}$  times the COARSE GAIN setting.

The instrument can be calibrated for a more precise current measurement, in which case a 1.00 megohm, 1% resistor is required. This resistor should be connected between the inner and outer conductors of the INPUT connector in place of the oxygen electrode. The controls are set as described in the last paragraph, with the exception that the COARSE GAIN is set to " $10^{-6}$ " for the calibration, and the ELECTRODE POLARIZATION is adjusted to provide a polarization voltage of 0.5000 volt. (The METER switch is temporarily set to "POLARIZATION VOLTAGE" for this adjustment, then returned to "OUTPUT"). The FINE GAIN is then adjusted so that a value of "50.0" is displayed. Unless the FINE GAIN control is moved, the instrument will now be fully calibrated for current measurements on all ranges as set by the COARSE GAIN control).

On the  $10^{-10}$  gain range, a slight offset from zero might be noticed due to leakage currents. With the ZERO CHECK switch set to "NORMAL" and nothing connected to the input, the FINE ZERO control can be adjusted to remove the offset.

#### D. OBTAINING OXYGEN MEASUREMENTS

Following calibration, leave the electrode hooked to the input of the Chemical Microsensor, remove it from the Calibration Cell, and insert it into the media material from which measurements are to be taken (Refer to your electrode manual



for special handling instructions).

If a reference electrode is needed (as with Transidynes microelectrode, Cat. #723, and needle electrode, Cat. #760), disconnect the calibration cell reference electrode from the Microsensor and connect the reference electrode to be used in the experiment (Transidyne Cat. #334 reference electrode with 1108 Microelectrode Holder). The reference electrode and working electrode need not necessarily be in close proximity, so long as there is electrical continuity between the two electrodes.

This means, for example, when working in a small animal, the reference electrode may be inserted beneath the skin, or under the tongue. WHEN USING A BIPOLAR ELECTRODE (CAT. #730, 731) DO NOT USE A REFERENCE ELECTRODE.

Note: Changing reference electrodes (from the one in the calibration cell to the one used in the tissue) will not affect your results. You may, however, wish to use the same reference throughout the operation, just for the sake of convenience.

If possible, the 21% calibration point should be checked about every 3 hours, at the conclusion of the experiment. Depending on the material or solution the electrode has been in contact with and the success of the initial stabilization, the contamination of the cathode can occur producing a slight drift. This is usually only a few percent per hour. If a calibration check indicates that drift has occurred, one can generally assume that the drift has been linear with time and make appropriate corrections in the measured values.

## 1. CORRECTION FOR WATER VAPOR PRESSURE

When any calibration instrument is used in which a gas mixture of known composition is dissolved in saline, the gas with which the saline is equilibrated will be saturated with water vapor. Since the water vapor dilutes the oxygen, resulting in a lower  $PO_2$ , the following correction should be made when doing a precision calibration.

(a) Calibration in mm Hg:

$$PO_2 \text{ (COR)} = \%O_2 \text{ (DRY)} (P_{\text{ATM}} - P_v) / 100$$

where  $PO_2 \text{ (COR)}$  = the corrected  $PO_2$  in the liquid to which the system should be calibrated

$\%O_2 \text{ (DRY)}$  = the percentage of oxygen in the dry gas mixture

$P_{\text{ATM}}$  = the current atmospheric pressure

$P_v$  = the vapor pressure as obtained from Table 3.1

(b) Calibration in  $\%O_2$ :

$$\%O_2 \text{ (COR)} = \%O_2 \text{ (DRY)} \left( 1 - \frac{P_v}{P_{\text{ATM}}} \right)$$

where  $\%O_2 \text{ (COR)}$  = the percentage of oxygen with respect to total dissolved gases.

A short listing of water vapor pressures at various temperatures is given in Table 3.1. For more information refer to the Handbook of Chemistry and Physics, published by the Chemical Rubber Co., under the heading "Vapor Pressure of Water below 100°C."

TABLE 3.1  
Vapor Pressure of Water

<u>Temp (°C)</u>	<u>V<sub>p</sub> (mm Hg)</u>
20.0	17.5
21.0	18.7
22.0	19.8
23.0	21.1
24.0	22.4
25.0	23.7
26.0	25.2
27.0	26.7
28.0	28.3
29.0	30.0
30.0	31.8
31.0	33.7
32.0	35.7
33.0	37.7
34.0	39.9
35.0	42.2
36.0	44.6
37.0	47.1
38.0	49.7
39.0	52.4
40.0	55.3

## IV. PROBLEM SOLVING

### A. LINEARITIES & STANDARD CURVE

Most oxygen electrodes will give a response which is within several percent of true linearity over a full range of  $PO_2$  (zero to atmospheric pressure). Other electrodes will not perform with as high a degree of linearity for reasons that are not well understood; however, by calibrating at a point in the vicinity of the expected  $PO_2$  (when a limited measurement range is being used) or by working from a standard curve (when a wide range is required), high accuracy can be achieved regardless of any electrode nonlinearity is observed is whether it is a reproducible characteristic of the electrode, a non-reproducible characteristic, or a calibration error.

Calibrating errors are primarily the result of insufficient stabilization, either prior to calibration or during calibration. If the electrode still has substantial long-term drift during calibration, neither the polarographic curve nor the standard curve will be accurate or reproducible. It is good practice to check the standard curve in both directions (high to low  $PO_2$  and vice versa) to confirm reproducibility. Review sections II, B and III, C 4(a) for information on long-term stabilization.

During the calibration procedure, it is necessary to observe the stabilization whenever the gas mixture is changed in order to make certain that the liquid in the calibration cell is fully equilibrated with the gas mixture. It is very important, particularly at very low or zero  $PO_2$  levels, that the gas flow is sufficiently high to displace all of the air which might otherwise come in contact with the liquid surface.

Flow sensitivity might be a cause of errors. After the electrode has stabilized at a particular gas flow rate, change the flow. If a change in polarographic current is observed,

it indicates that the electrode is flow sensitive, in which case the calibration must be done with gas shut off and fluid motionless (See Section III, C 4(b)).

Temperature instability can also produce apparent non-linearity. Use a high-resolution thermometer to confirm that the temperature is not drifting. It should be stable to within several tenths of a degree Celcius.

An unstable type of electrode nonlinearity can be caused by a poor electrode design or faulty construction in which oxygen is either absorbed into the insulating material around the cathode or else the electrolyte seeps between the cathode and insulation. In such a case, a loss of sensitivity will appear at low  $PO_2$  levels, since oxygen will be diffused from the insulation to the cathode, producing an erroneously high oxygen current. The absorbed oxygen will in time become depleted at low  $PO_2$ , resulting in a shift in the calibration. To check for this condition, make certain that the residual current does not show excessive downward drift when maintaining a zero  $PO_2$  environment.

#### B. HIGH RESIDUAL CURRENT

The residual current at zero oxygen levels should generally be less than five <sup>(5)</sup> percent of the current obtained at 21 percent oxygen. If the percentage is greater, the sensitivity of the electrode may be insufficient to produce a noise-free, stable response.

Excessive residual currents usually result from defective electrodes with leaky insulation. Before discarding the electrode, however, it is recommended that the Chemical Microsensor and electrode connecting cable be checked as alternative sources of leakage current. The electrode should be disconnected, leaving the cable attached to the instrument with polarization voltage applied. If the current does not drop substantially

from the residual value, one should suspect that the cable or instrument are defective. Next, disconnect the cable from the instrument. If the current is still high, the problem is in the instrument (refer to Section VI). Otherwise, replace the cable or electrode as appropriate.

### C. DRIFT

As explained in Section II, B, all oxygen electrodes will drift upon initial use. If the electrode does not stabilize after over 48 hours of polarization in saline, one should assume that it needs to be repaired or replaced. If it is stable in saline but drifts when used in another medium, the electrode is apparently attracting proteins or other molecules which change its sensitivity. A long polarization period in this medium prior to calibration will usually stabilize the current.

Membranes over the cathode usually help to reduce drift by keeping contaminants off the active surface. If the electrode does not already have a membrane, one can often be applied by dipping the electrode in appropriate solutions such as collodion. Refer to the instructions which came with the electrode for more information.

When Ringers type solutions containing a variety of ions and pH buffers have been used, polarographic currents have been observed to drift continually upward, with no apparent stabilization. Subsequent microscopic examinations revealed large opaque deposits over the cathode. These deposits are suspected to be calcium carbonate or other compounds which have precipitated out of solution. For these solutions to remain stable and usable with micro-oxygen electrodes, the proper pH must be maintained at all times by control of the dissolved  $\text{CO}_2$ .

#### D. EXCESSIVELY HIGH CURRENT

When using micro-oxygen electrodes, the sudden appearance of unusually high currents is an indication that the electrode tip is broken. These electrodes should always be inserted in tissue using a micromanipulator, keeping all forces parallel to the axis of the electrode. Also, avoid gas flow directly over the electrode tip in the Calibration Cell.

#### E. NOISE & STATIC CHARGE

Some electrodes will be excessively noisy when new and first polarized; however, the noise will decrease when the electrode is sufficiently stabilized. Noise in the Calibration Cell can be caused by excessive turbulence, with high gas-flow rates and bubbles adhering to the electrode tip. Cyclic current variations over a period of seconds or minutes might be due to poor temperature regulation.

Another type of noise can be caused by electrostatic fields. Movement of people or objects near the electrode will sometimes produce large transient current fluctuations. Grounding of the electrode holder and micromanipulator will usually correct this problem. In extreme cases, it might be necessary to use a Faraday cage as a shield.

V  
CIRCUIT DESCRIPTION

With reference to the functional diagram in Figure 4.1, the operation of the Chemical Microsensor is as follows:

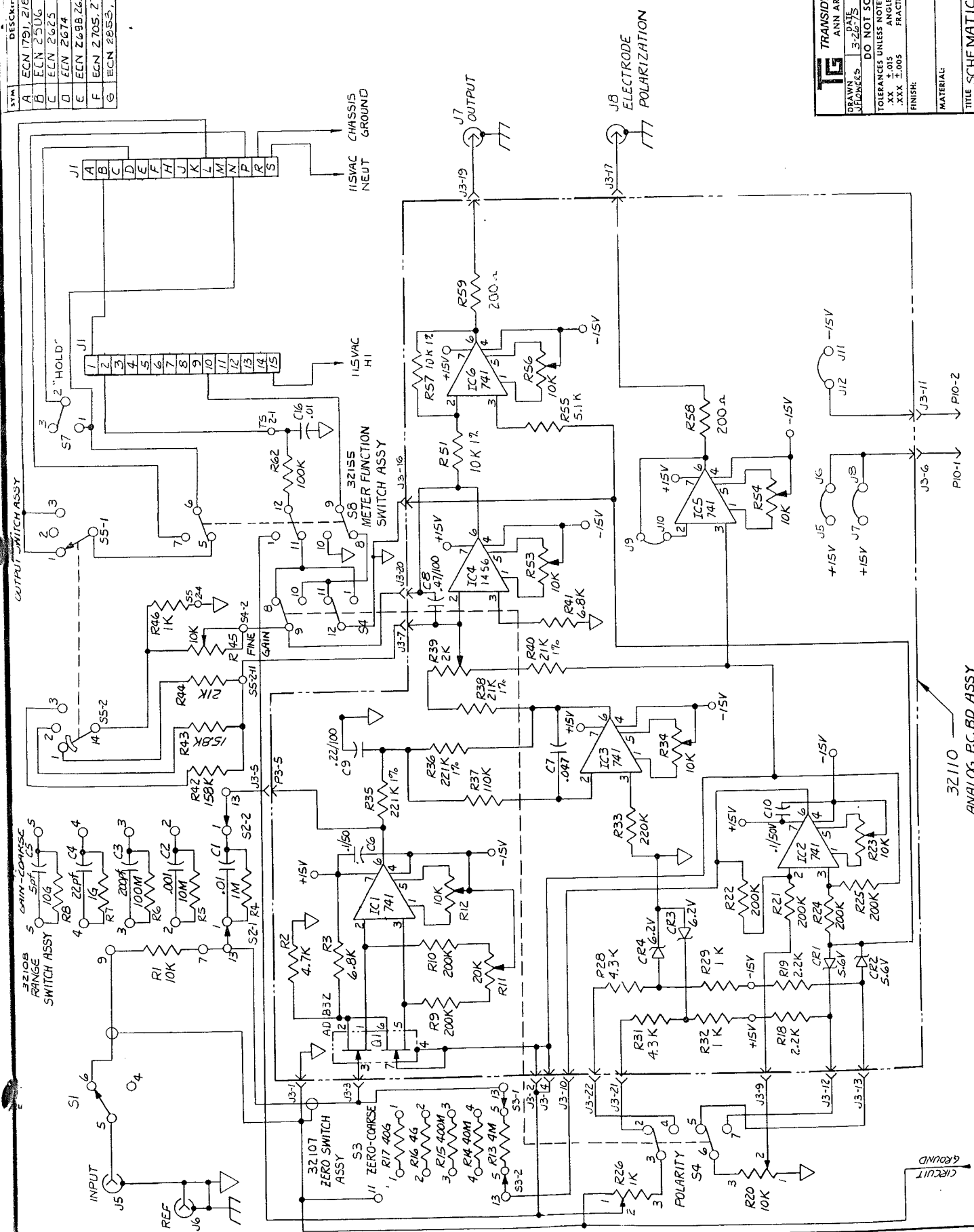
1. Input is the back panel BNC input connector.
2. Reference is the back panel BNC reference connector.
3. Amp. 1 is a current-to-voltage converter with variable gain controls.
4. Zero Current is a current source to compensate for residual current flow during measurement of zero concentration of a chemical species.
5. Elect. Polar. is a voltage source to provide electrode polarization voltage.
6. Filter is a 10-Hz low bandpass filter at 40 decibels per frequency decade rolloff.
7. Amp. 2 is a differential amplifier which subtracts the electrode polarization from the output of the current-to-voltage converter.
8. Amp. 3 and Amp. 4 provide single-ended analog outputs for electrode current and electrode polarization, respectively.
9. Meter is the digital display.

For additional information, see the circuit diagrams in Figures 4.2 and 4.3



Figure 4.1. Chemical Microsensor Functional Diagram

SYM	DESCRIPTION	DATE	APP'D
A	ECN 1791, 2/184	2/75	11
B	ECN 2506	2-278	11
C	ECN 2625	11-1076	11
D	ECN 2674	11/74	11
E	ECN 2698B, 2700	2/77	11
F	ECN 2705, 2704	1/5/77	11
G	ECN 2853, 2754	5-2-77	11



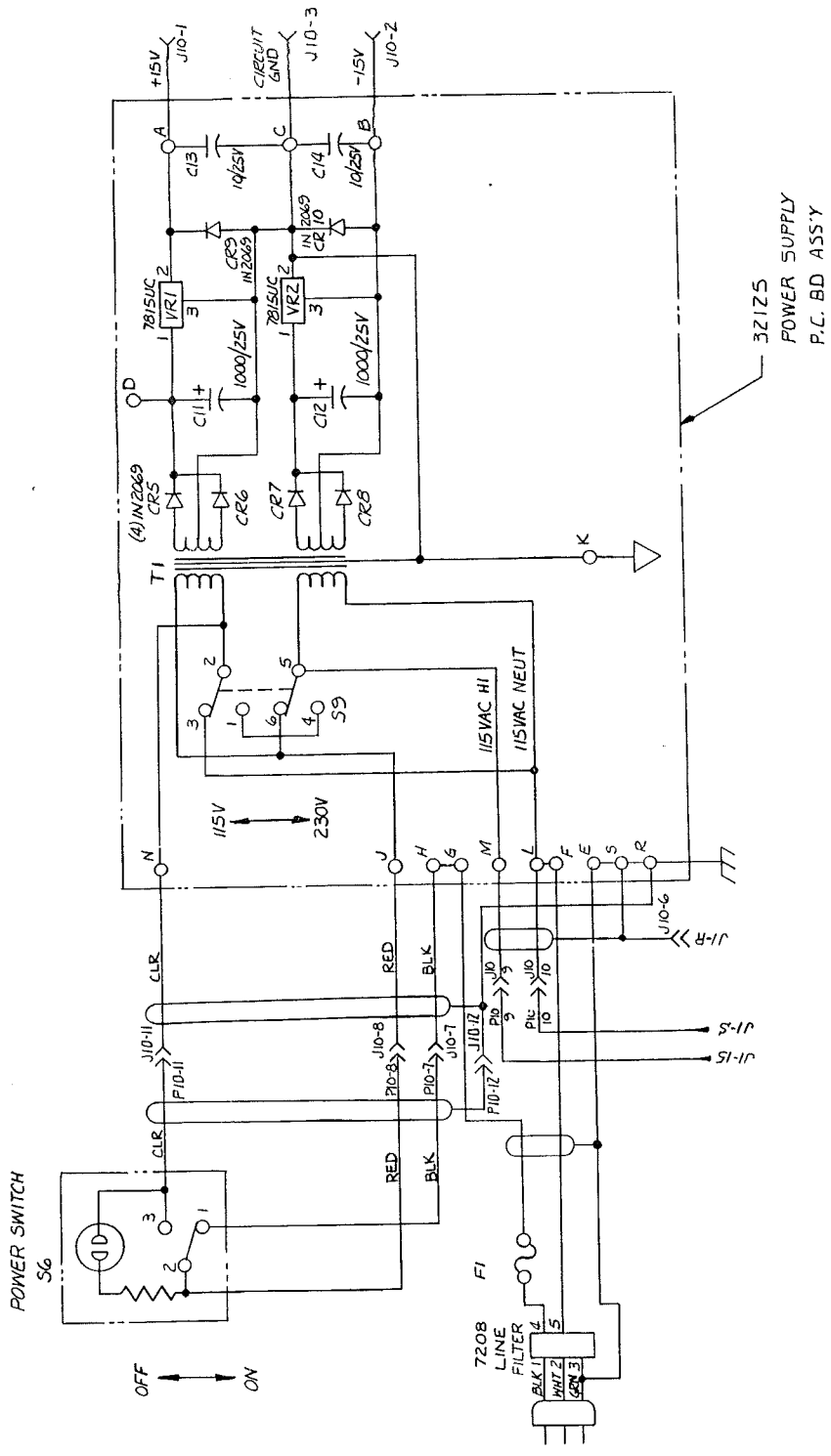
1201

<b>TG</b> TRANSIDYNE GENERAL CORP.	
ANN ARBOR, MICH. 48103	
DATE	SCALE
3-2-75	APPROVED
DO NOT SCALE DRAWING	
TOLERANCES UNLESS NOTED	FINISH
XX ±.015	FRACTION ±1°
XXX ±.005	FRACTION ±1/64
MATERIAL	
TITLE	
SCHEMATIC DIAGRAM	
N/A	DWG. NO.
1201	1201
PRODUCT	REV.
1201	6

Figure 4.2. Schematic Diagram of Chemical MicroSensor

1201

SYMBOL	DESCRIPTION	DATE	APPROVED
--------	-------------	------	----------



3Z1Z5  
POWER SUPPLY  
P.C. BD ASS'Y

SYM	LAST NO.	LAST NO.	OMITTED
C	16	15	
CR	10		4
J	12		
R	62	21,30,47-50,52,60	
S	9		

Figure 4.3. Schematic Diagram of Chemical MicroSensor

<b>TRANSIDYNE GENERAL CORP.</b> ANN ARBOR, MICH. 48103			
DRAWN	3-2-65	SCALE	APPROVED
CHECKED			
DO NOT SCALE DRAWING			
TOLERANCES UNLESS NOTED			
XXX	±.05	ANGLE	±1°
XXX	±.005	FRACTION	±1/64
FINISH:			
MATERIAL:			
TITLE SCHEMATIC DIAGRAM			
OZ METER			
N/A	DWG. NO.	REV.	F
PRODUCT	1201		

## VI

### INSTRUMENT TEST PROCEDURE

When a proper response cannot be obtained from the Chemical Microsensor System, the problem is almost always in the electrode. If the procedures described in sections III (B) through IV do not reveal the source of difficulty, an instrument malfunction should be suspected.

Before returning an instrument for service, the user should perform several tests to determine whether the instrument is actually malfunctioning or whether the problem lies elsewhere.

First, the current-sensing amplifier and internal zeroing current source should be checked. (See the circuit diagrams in Section VI). To do so, all cables should be disconnected from the rear panel, and the controls initially set as follows:

POWER switch on  
METER switch to output  
OUTPUT switch to  $\%$   
COARSE GAIN to  $10^{-6}$   
FINE GAIN full counterclockwise  
COARSE ZERO to  $10^{-8}$   
FINE ZERO full counterclockwise  
ZERO CHECK to normal  
ELECTRODE POLARITY to "-"  
ELECTRODE POLARIZATION full counterclockwise

With these steps taken, the test is ready to be performed. Any deviation from the indicated response is a sign of possible instrument malfunction.

(1) With the controls set as indicated above, the instrument should display a value of  $\pm 0.5$ .

(2) Turn the FINE ZERO control to its full clockwise position. The displayed value should be greater than -100.0 (NOTE: the negative sign is present in the display as a result of the polarity of the internal current source). Return the FINE ZERO control to its original position.

(3) Set the FINE ZERO so that a value of -5.0 is displayed. Turn the FINE GAIN to its full clockwise position. The displayed value should be greater than -50.0. Return the FINE GAIN to its original position; the display should return to -5.0.

(4) Set the COARSE GAIN to  $10^{-7}$ . The display should change from -5.0 to  $-50.0 \pm 5.0$ .

(5) Set the COARSE ZERO to  $10^{-9}$ . The displayed value should be  $-5.0 \pm 0.5$ .

(6) Set the COARSE GAIN to  $10^{-8}$ . The displayed value should be  $-50.0 \pm 5.0$ .

(7) Set the COARSE ZERO to  $10^{-10}$ . The displayed value should be  $-5.0 \pm 0.5$ .

(8) Set the COARSE GAIN to  $10^{-9}$ . The displayed value should be  $-50.0 \pm 5.0$ .

(9) Set the COARSE ZERO to  $10^{-11}$ . The displayed value should be  $-5.0 \pm 0.5$ .

(10) Set the COARSE GAIN to  $10^{-10}$ . The displayed value should be  $-50.0 \pm 5.0$ .

NOTE: If some of the displayed values are slightly out of the specified tolerances, it is an indication of drift resulting from the aging of the range-selecting resistors. This is not a serious malfunction and will cause no problem unless absolute current measurements based on a precision calibration at a different COARSE GAIN setting are being made.

(11) Set the COARSE ZERO to  $10^{-12}$ . Adjust the FINE ZERO so that the display reads 0.0. If this setting can be made, increase the FINE ZERO to its full clockwise position. A value greater than -100.0 should be displayed.

(12) Reset the FINE ZERO so that 0.0 is displayed. Turn the ELECTRODE POLARIZATION control to its full clockwise position. The displayed value should be less than 10.0.

(13) Set the METER switch to ELECTRODE POLARIZATION. The display should indicate a value greater than -1.000.

For the remaining tests, a voltmeter with a high input impedance (at least 11 megohms) is required as well as appropriate connectors for attaching the voltmeter to the BNC connectors on the Chemical Microsensor.

(14) Connect the voltmeter to the INPUT connector. Set the ELECTRODE POLARIZATION control so that the display indicates 1.000. The voltmeter should read 1.0 volt. Switch the COARSE GAIN control through all of its settings. The voltmeter should continue to read 1.0 volt.

(15) Rotate the ELECTRODE POLARIZATION control to its full counterclockwise position. The voltmeter should indicate 0.0 volt on all settings of the COARSE GAIN control.

(16) Set the METER switch to OUTPUT and adjust the FINE ZERO control for a displayed value of -100.0. Connect the voltmeter to the OUTPUT connector. The voltmeter should indicate a value of -1.0 volt.

If the Chemical Microsensor performs adequately on these tests, the instrument is in proper working order, and the difficulty is external to the instrument. Make certain that all of the connecting cables have appropriate continuity and are not short circuited.

If the instrument fails any of these tests, or if the difficulty cannot be located, please contact our Service Department (SEE Section VII).

## INSTRUMENT SERVICE INFORMATION

If you believe that service is required on your Chemical Microsensor, please phone our Service Department before returning the instrument. If the instrument must be shipped to Transidyne General, attach a tag identifying the owner and the problem encountered. In any correspondence regarding an instrument, always specify the model and serial numbers.

When returning an instrument, use the original packaging materials, if possible. If these are not available, pack the instrument as follows:

- (1) Wrap the instrument in heavy paper or plastic.
- (2) Place the instrument in a heavy carton with padding on all sides.
- (3) Seal with strong tape.
- (4) Mark the container "Delicate Instrument" or "Fragile."
- (5) Ship to:

Transidyne General Corporation  
Service Department  
3850 Research Park Drive  
Ann Arbor, MI 48104  
Phone: (313) 769-1900

## OXYGEN MEASUREMENT BIBLIOGRAPHY

The following bibliography contains information pertaining to polarographic oxygen measurements. The articles are categorized as follows:

- I. General Information
- II. Microelectrodes
- III. Applications (Note specific use)



DIAMOND ELECTRO-TECH, INC.

Instructions  
For The Use Of  
Type 760 Oxygen Electrodes

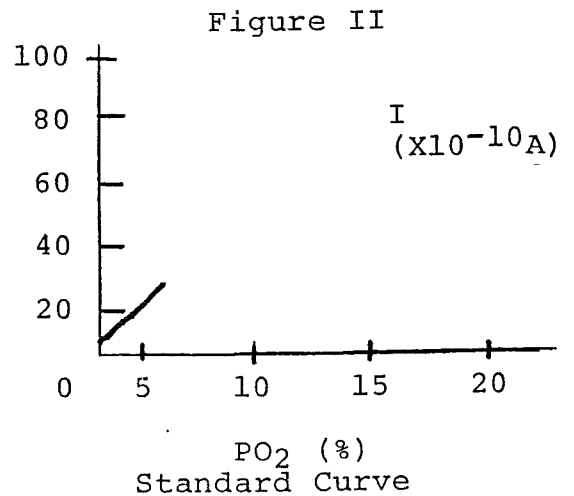
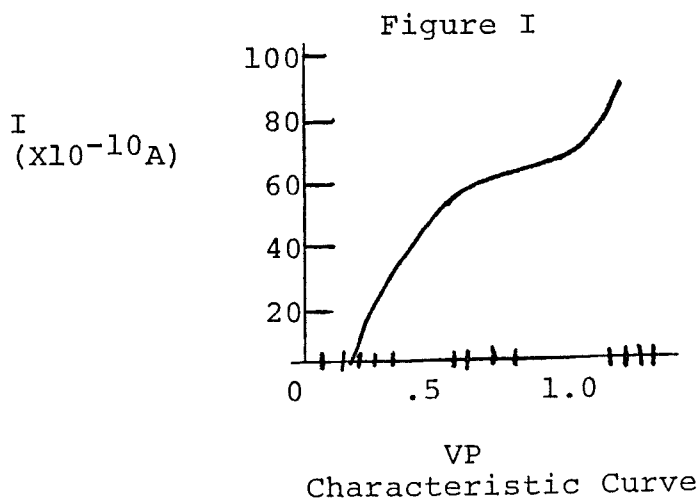
IMPORTANT: Please read these instructions, even if you have used our electrodes in the past. We are continually striving to upgrade our electrodes and use procedures, and these instructions may differ from those published in your Chemical Microsensor Manual.

ELECTRODE CHARACTERISTICS:

Even though we might ideally like to have pre-calibrated, drift-free oxygen electrodes, achieving such performance has always been very difficult when using miniature electrodes with biological materials. The reason is that with small cathode surface areas (in this case about 500  $\mu^2$ ), the contamination of the cathodic surface by proteins and other large molecular structures can change its ability to reduce oxygen and thus alter the calibration. Fortunately, there are several things which the user can do to stabilize the electrode, and with an understanding of several key parameters, the electrode will prove to be an accurate and convenient means of obtaining  $PO_2$  measurements which would not otherwise be possible.

When a cathode, made of a noble metal, is polarized with a negative potential of less than one volt in a solution containing electrolytes and dissolved oxygen, current will flow due to the reduction of oxygen at the cathodic surface. Although solutes other than oxygen can be reduced, these reactions require a higher polarization voltage than oxygen.

The voltage-current relationship of the oxygen electrode is called the characteristic curve. Figure 1 is a typical example. As the polarization voltage is increased above approximately 0.5 volt, the current will tend to reach a plateau. In the plateau region, the current is limited by the rate at which oxygen can diffuse to the cathode and will be directly proportional to the  $PO_2$ , as shown in the standard curve of Figure 2. As the polarization voltage is increased beyond 1.0 volt, other elements begin to react at the cathode and the current rises rapidly.



The shape of the characteristic curve can vary considerably between electrodes and also changes with 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 a midpoint occurring anywhere between 0.5 and 0.95 volt.

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. Occasionally the characteristic curve might vary considerably from the classic and have a poorly defined plateau, but we have not observed that the standard curve is simultaneously degraded.

It should be noted that in the standard curve of Figure 2, the current at zero  $PO_2$  is not zero. This is called the "dark current" and is typically observed on oxygen electrodes with extremely small cathode areas. It can result from reduction of oxygen and various impurities which are present in the materials surrounding the cathode and from electrical leakage through various insulating materials used in the system. The ZERO controls on the Chemical Microsensor can be adjusted to compensate for this dark current so that it does not appear in the readings when the instrument is calibrated to indicate  $PO_2$  directly.

All oxygen electrodes tend to drift, particularly upon initial operation. Drift is particularly apparent in solutions containing proteins, and even more so in the presence of hemoglobin. It occurs as a result of large molecules attaching themselves to the cathode, changing its surface area and operating characteristics. The use of a membrane will protect the cathodic surface from direct contact with interfering molecules and thus substantially reduce the drift, though not necessarily eliminate it.

Drift is minimized by "aging" the electrode while polarized in the type of solution in which  $PO_2$  measurements are to be made. After several hours of operation, the electrode will usually stabilize and thereafter drift only a few percent.

Temperature is an additional consideration. Since diffusion rates vary with temperature, so will the calibration of the oxygen electrode. Therefore, it is necessary to calibrate the electrode at the same temperature as that of the experimental subject or material.

## CALIBRATION AND USE PROCEDURES:

### Initial Aging and Use of Membranes:

As explained in the previous section, an oxygen electrode will drift as contaminants build up on the cathodic surface. A membrane which is permeable to both oxygen and electrolytes is sometimes used to help reduce drift, both by protecting the cathode and by increasing the surface area on which contamination can take place. Ideally, most of the diffusion gradient will occur within the membrane, so the gradient at the outer surface of the membrane will be very small.

The Type 760 oxygen electrode is not shipped with any membrane coating, the reason being that the electrode is designed as a durable, non-disposable electrode which should give you years of use, while most membrane materials have a limited use life. Furthermore, many applications do not require membranes.

As the bare cathode is polarized in biological solution, the current will decrease as contaminants (usually proteins) collect on the cathodic surface. However, as this build-up of contaminants increases, it tends to behave as a membrane and the electrode becomes stable. Many users find that preconditioning the bare cathode in a solution similar to that in which  $PO_2$  measurements will be made is adequate.

Numerous membrane formulations have appeared in the literature. One popular and readily available material is collodion. The tip of the electrode is dipped in the material and allowed to dry. The solution should be sufficiently dilute so that a large drop of material does not remain on the tip; otherwise, an excessively long response time might result.

Regardless of whether or not a membrane is used, the electrode will require preconditioning prior to calibration. To precondition the electrode, polarize it with a potential of -1.0 volt in a solution similar to that in which the  $PO_2$  measurements will be made. Leave it in this solution for a period of two to four hours. If the temperature and  $PO_2$  of the solution are constant, the current through the electrode will tend to stabilize. The electrode is then ready to calibrate.

### Cleaning and Reconditioning Electrode Tips:

The electrode can be cleaned with most cleaning solutions and membrane materials can be removed with appropriate solvents. The resin used in making the electrode is resistant to most solvents, though we do not recommend soaking it for long periods of time.

The cathode can be reconditioned by lightly hand-polishing the tip with a hard, fine, Arkansas Stone. This procedure is recommended if normal current levels are not observed after cleaning or when solvent resistant membranes have been applied.

Selection of Polarization Voltage and Production of Characteristic Curve:

Most Type 760 Electrodes will function well when polarized with a potential of -0.80 volt. However, we recommend that for optimum performance, the user produce a characteristic curve and thus choose the most appropriate polarization voltage.

To produce a characteristic curve, the Chemical Microsensor (or other instrumentation) must be set up to indicate current. Most of the electrodes operate in a current range between  $10^{-10}$  and  $10^{-8}$  ampere.

On the Model 1201, the units on the COURSE GAIN control indicate the full scale current when the OUTPUT control is set to % and the FINE GAIN, COURSE ZERO and FINE ZERO controls are all at their maximum counterclockwise positions. Therefore, when the COURSE GAIN is set to  $10^{-8}$ , the full scale current (when the readout indicates 100.0%) is  $1 \times 10^{-8}$  ampere, and the display should be multiplied by  $10^{-10}$  to obtain actual current.

The beaker in the Calibration Cell should be filled with about 80 ml of 0.9% NaCl, which has been allowed to equilibrate with the gasses in room air (21% oxygen). The temperature should be set to that at which the electrode will be used.

Immerse the electrode tip in the solution and set the polarization voltage to an initial value of -1.0 volt. The current through the electrode will take at least several minutes to stabilize. A strip chart recorder (with a 1.0 volt full scale sensitivity) connected to the output of the Model 1201 will aid in observing the electrode stabilization. When the electrode has stabilized at -1.0 volt, record the current and decrease the polarization voltage to -0.9 volt. Repeat the procedure at 0.1 volt intervals, allowing the electrode to stabilize at each level. At some value below -0.5 volt, the current may reverse; this is normal and the procedure should be discontinued at this point.

After plotting the characteristic curve, observe 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. As the electrode ages, the shape of the characteristic curve will vary; however, the operating voltage chosen initially will usually remain in the plateau region.

### Calibration of Chemical Microsensor and Production of Standard Curve:

The simplest type of calibration, which does not require plotting a standard curve, involves using 21% O<sub>2</sub> (air) and 0% O<sub>2</sub> (100% nitrogen) to produce two calibration points. This procedure is described in the Chemical Microsensor Manual and involves adjusting the instrument to read directly in units of partial gas pressure. 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 nitrogen should always be used to determine the zero point and thereby allow compensation for the dark current.

The ultimate accuracy is achieved by plotting a standard curve, which relates PO<sub>2</sub> to current. In this case, the Chemical Microsensor is used as a current meter and PO<sub>2</sub> is determined from the standard curve.

To make a standard curve, several tanks of oxygen-nitrogen mixture (usually 5% and 10% oxygen) are required, as well as 100% nitrogen and air. The Chemical Microsensor is set up as a current meter as described previously. Beginning with air, the gas is bubbled through the calibration cell containing the electrode until a steady current indicates equilibrium has been reached and the final current is recorded. Then the gas mixture with next lowest oxygen concentration is bubbled through the calibration cell and the procedure is repeated until 100% nitrogen has been used. The recorded current values, when plotted against their respective PO<sub>2</sub> values, produce the standard curve.

### Drift:

Regardless of the calibration method chosen, it is important to realize that some electrodes 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 experimental design will allow for it, additional calibration checks during the experiment would be advisable.

### Noise:

The noise level of the electrode is sufficiently low so that it will not interfere with accurate PO<sub>2</sub> 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 always 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.

In Case of Difficulty:

If you have problems in using these electrodes, please feel free to contact us. These electrodes are still of an experimental nature, but with proper use they will provide a convenient and accurate means of producing PO<sub>2</sub> measurements which would otherwise not be possible.

Also, a few of our earlier Chemical Microsensors (Model 1201) developed internal noise problems. If you suspect that your instrument is not functioning properly, please contact our Electrophysiology Department for some simple test procedures.

WARRANTY:

Any electrode which upon initial use (with no membrane) fails to produce an identifiable plateau (as defined in these instructions) in 0.9% NaCl having a PO<sub>2</sub> of 160 mm Hg (21%) at 37° C will be replaced, providing the electrode is returned to us within six (6) months of shipping date. We cannot guarantee the performance after a membrane has been applied or the tip reconditioned.

IMPORTANT NOTE:

THESE ELECTRODES ARE NOT FOR HUMAN USE!

/amm  
2-10-77  
P/N 90174

INSTRUCTIONS  
FOR THE USE OF  
TYPE, 723 OXYGEN ELECTRODES

IMPORTANT:

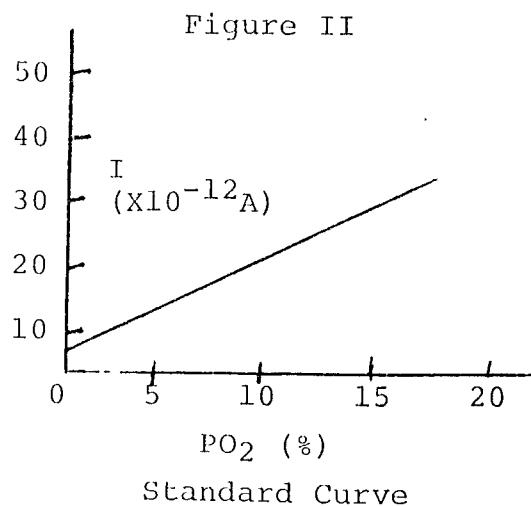
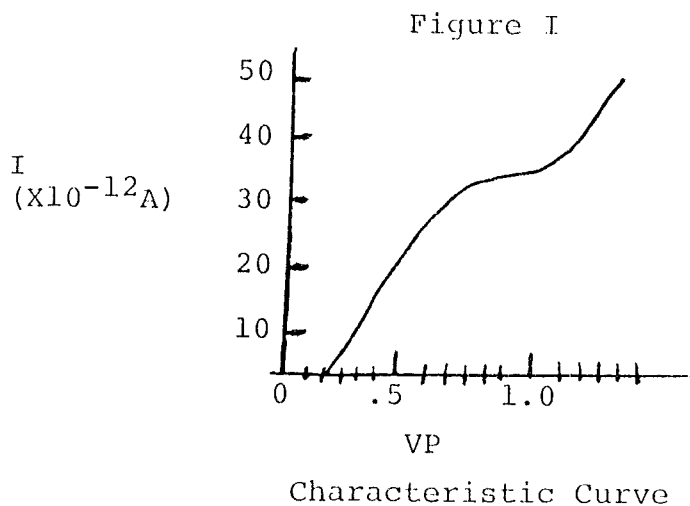
Please read these instructions, even if you have used our electrodes in the past. We are continually striving to upgrade our electrodes and use procedures - and these instructions may differ from those published in your Chemical Microsensor Manual.

ELECTRODE CHARACTERISTICS:

Even though we might ideally like to produce all of our electrodes with identical characteristics - such has not been possible due to small variations in the tips (which are hand-beveled) and in plating the micro cathodes. Thus; the user must calibrate his instrument for each electrode and an understanding of several key parameters is essential to obtaining accurate PO<sub>2</sub> measurements.

When a cathode, made of a noble metal, is polarized with a negative potential of less than one volt in a solution containing electrolytes and dissolved oxygen, current will flow due to the reduction of oxygen at the cathodic surface. Although solutes other than oxygen can be reduced, these reactions require a higher polarization voltage than oxygen.

The voltage-current relationship of the oxygen electrode is called the characteristic curve. Figure I is a typical example. As the polarization voltage is increased above approximately 0.5 volt, the current will tend to reach a plateau. In the plateau region, the current is limited by the rate at which oxygen can diffuse to the cathode and will be directly proportional to the PO<sub>2</sub> as shown in the standard curve of Figure II. As the polarization voltage is increased beyond 1.0 volt, other elements begin to react at the cathode and the current rises rapidly.



The shape of the characteristic curve can vary considerably between electrodes and also changes with shelf life. 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 a midpoint occurring anywhere between 0.5 and 0.95 volt.

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. Occasionally the characteristic curve might vary considerably from the classic and have a poorly defined plateau, but we have not observed that the standard curve is simultaneously degraded.

It should be noted that in the standard curve of Figure II the current at zero  $PO_2$  is not zero. This is called the "dark current" and is typically observed on oxygen electrodes with extremely small cathode areas. It can result from reduction of oxygen and various impurities which are present in the materials surrounding the cathode and from electrical leakage through various insulating materials used in the system. The ZERO controls on the Chemical Microsensor can be adjusted to compensate for this dark current so that it does not appear in the readings when the instrument is calibrated to indicate  $PO_2$  directly.

All oxygen electrodes tend to drift, particularly upon initial operation. Drift is particularly apparent in solutions containing proteins, and even more so in the presence of hemoglobin. It occurs as a result of large molecules attaching themselves to the cathode, changing its surface area and operating characteristics. The use of a membrane will protect the cathodic surface from direct contact with interfering molecules and thus substantially reduce the drift, though not necessarily eliminate it.

\*Drift is minimized by "aging" the electrode while polarized in the type of solution in which  $PO_2$  measurements are to be made. After several hours of operation, the electrode will usually stabilize and thereafter drift only a few percent.

Temperature is an additional consideration. Since diffusion rates vary with temperature, so will the calibration of the oxygen electrode. Therefore, it is necessary to calibrate the electrode at the same temperature as that of the experimental subject or material.



CALIBRATION AND USE PROCEDURES:

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 gasses 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.

Selection of Polarization Voltage and Production of Characteristic Curve:

Most Type 723 Electrodes will function well when polarized with a potential of -0.75 volt. However, we recommend that for optimum performance, the user produce a characteristic curve and thus choose the most appropriate polarization voltage.

To produce a characteristic curve, the Chemical Microsensor (or other instrumentation) must be set up to indicate current in picoamperes. This can be done on the Model 1201 by setting the OUTPUT switch to %, the COURSE GAIN to  $10^{-10}$ , the COURSE ZERO to  $10^{-12}$ , and both the FINE GAIN and FINE ZERO to their full counter-clockwise positions. The display will then indicate current in picoamperes ( $I \times 10^{-12}$  amp).

The beaker in the Calibration Cell should be filled with about 80 ml of 0.9% NaCl, which has been allowed to equilibrate with the gasses in room air (21% oxygen). The temperature should be set to that at which the electrode will be used.

Carefully lower the electrode into the solution and set the polarization voltage to an initial value of -1.0 volt. The current through the electrode will take at least several minutes to stabilize. A strip chart recorder (with a 1.0 volt full scale sensitivity) connected to the output of the Model 1201 will aid in observing the electrode stabilization. When the electrode has stabilized at -1.0 volt, record the current and decrease the polarization voltage to -0.9 volt. Repeat the procedure at 0.1 volt intervals, allowing the electrode to stabilize at each level. At some value below -0.5 volt, the current may reverse: this is normal and the procedure should be discontinued at this point.

After plotting the characteristic curve, observe the plateau, defined as the region of minimum slope. The voltage at the mid-

point of the plateau should be chosen as the operating voltage for the electrode. As the electrode ages, the shape of the characteristic curve will vary; however, the operating voltage chosen initially will usually remain in the plateau region.

#### Calibration of Chemical Microsensor and Production of Standard Curve:

Before producing a standard curve (or calibrating the Chemical Microsensor), the electrode should be polarized in a solution similar to that in which measurements will be made for at least several hours. If the solution is stable with respect to  $PO_2$  and temperature, the stabilization can be observed on a strip chart recorder.

The simplest type of calibration, which does not require plotting a standard curve, involves using 21%  $O_2$  (air) and 0%  $O_2$  (100% nitrogen) to produce two calibration points. This procedure is described in the Chemical Microsensor Manual and involves adjusting the instrument to read directly in units of partial gas pressure. 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 and thereby allow compensation for the dark current.

The ultimate accuracy is achieved by plotting a standard curve, which relates  $PO_2$  to current. In this case, the Chemical Microsensor is used as a current meter and  $PO_2$  is determined from the standard curve.

To make a standard curve, several tanks of oxygen-nitrogen mixture (usually 5% and 10% oxygen) are required, as well as 100% nitrogen and air. The Chemical Microsensor is set up as a current meter as described previously. Beginning with air, the gas is bubbled through the calibration cell containing the electrode until a steady current indicates equilibrium has been reached and the final current is recorded. Then the gas mixture with next lowest oxygen concentration is bubbled through the calibration cell and the procedure is repeated until 100% nitrogen has been used. The recorded current values, when plotted against their respective  $PO_2$  values, produce the standard curve.

#### Drift:

Regardless of the calibration method chosen, it is important to realize that some electrodes 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 experimental design will allow for it, additional calibration checks during the experiment would be advisable.

Noise:

The noise level of the electrode is sufficiently low that it will not interfere with accurate PO<sub>2</sub> 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 impedences involved. Good grounding and shielding practices should always 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.

In Case of Difficulty:

If you have problems in using these electrodes, please feel free to contact us. These electrodes are still of an experimental nature, but with proper use they will provide a convenient and accurate means of producing PO<sub>2</sub> measurements which would otherwise not be possible.

Also, a few of our earlier Chemical Microsensors (Model 1201) developed internal noise problems. If you suspect that your instrument is not functioning properly, please contact our Electrophysiology Department for some simple test procedures.

WARRANTY:

Any electrode which upon initial use fails to produce an identifiable plateau (as defined in the previous instructions) or fails to produce a current of at least 5 picoamperes ( $5 \times 10^{-12}$  amp) when polarized according to these instructions in a 0.9% NaCl solution having a PO<sub>2</sub> of 160 mm Hg (21%) and a temperature of 37<sup>0</sup> C will be replaced, providing the electrode is used within six (6) months of shipping date and is returned with tip intact. (Please include lot number when returning any electrode).

IMPORTANT NOTE:

THESE ELECTRODES ARE NOT FOR HUMAN USE!



TRANSIDYNE GENERAL INSTALLATION FORM

In order to insure you the customer of quality service, each section of the operation manual should be read before attempting the actual installation. Become familiar with all the terms and aids available in the manual. Do not start the instrument until you have read the manual.

The following is a checklist of each section in the manual. Only check an item after you have completely covered that section. Your manual has been set up to give you a step by step progression on how to operate your instrument; follow it! (If salesrepresentative installs instrument check-off list still applies).

- 1. Product information \_\_\_\_\_
- 2. Installation Procedures \_\_\_\_\_
- 3. User Orientation \_\_\_\_\_
- 4. Operating Instructions \_\_\_\_\_
- 5. Operational Precautions \_\_\_\_\_
- 6. Operational Hazards \_\_\_\_\_
- 7. Problem Solving \_\_\_\_\_
- 8. Repair and Service \_\_\_\_\_
- 9. Service Contract \_\_\_\_\_
- 10. Service Manual \_\_\_\_\_

I have reviewed all of the information and installation has been completed to my satisfaction.

Name: \_\_\_\_\_  
Institution: \_\_\_\_\_  
Address: \_\_\_\_\_  
\_\_\_\_\_

Company Representative: \_\_\_\_\_

(If installed by representative)

Signature: \_\_\_\_\_  
Date: \_\_\_\_\_

Signature: \_\_\_\_\_  
Date: \_\_\_\_\_

Your warranty is not in effect if this form has not been completed. Please return this form and warranty card immediately upon installation.

Dr. Barry Feinberg  
Kendall Research Center  
411 Lake Zurich Road  
Barrington, IL 60010

CALIBRATION CELL

MODEL 1251

INSTRUCTION MANUAL

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## I. GENERAL DESCRIPTION

### 1.1 Description

The new Calibration Cell Model 1251 has a number of new and improved features. It was designed so that the researcher will be able to conveniently and rapidly calibrate oxygen or hydrogen sensing electrodes with several gas concentrations.

It features a small beaker of 100 ml so that fluid rapidly equilibrates with gas. Its glass construction prevents contamination from construction materials. The temperature is precisely controlled to match the temperature of the experimental situation.

It has a 4-way distribution valve so four different calibrating tanks can be permanently connected to the Calibration Cell. Thus, the researcher need only to valve to the appropriate tank to bubble the gas into the beaker.

### 1.2 Specifications

Temperature Range:	+10°C to 60°C
Control Accuracy:	±0.1°C
Tank Capacity:	1 gallon
Tank Dimensions:	9.75 x 5.0 x 7.0 inches
Immersion Heater:	500 watts
Built-in Stirrer:	Provides constant agitation of liquid medium.
Weight:	7 pounds
Electrical Requirement:	115 VAC (230 VAC available).

## II. INSTALLATION

### 2.1 Initial Inspection

This instrument was carefully inspected both mechanically and electrically before shipment. The instrument should be inspected for physical damage in transit and performance tested as per Section III, OPERATING INSTRUCTIONS before use.

### 2.2 Power Requirement

115 VAC at 50 - 60 Hz (230 VAC at 50-60 Hz available)

### 2.3 Installation

2.3.1 Fill water tank to 1/2" of top.

2.3.2 Place temperature regulator at shallow end of water tank. This end of the water tank allows for better mixing of water by stirrer.

2.3.3 Place water tank cover on water tank.

2.3.4 Fill glass beaker with saline or other appropriate fluid.  
*80 ml of 0.9% NaCl*

2.3.5 Insert L-shaped rod in holder and secure thumb screw.

2.3.6 Slide clamp onto L-bracket and insert electrode holder into clamp. Tighten the thumb screws.

### 2.4 Repair and Service

If the instrument is to be shipped to Transidyne General for service or repair, attach a tag to the instrument identifying the owner and indicating the repair to be accomplished. In any correspondence, identify the instrument by model number.

Return to:

Service Department  
TRANSIDYNE GENERAL CORPORATION  
3850 Research Park Drive  
Ann Arbor, MI 48104 U.S.A.



2.5 Repackaging for Service.

- a. Wrap the instrument in heavy paper or plastic.
- b. Place instrument in a heavy carton and arrange packaging material around all sides of the unit.
- c. Seal with strong tape.
- d. Mark shipping container with "Delicate Instrument," "Fragile," etc.

III. OPERATING INSTRUCTIONS

3.1 Calibration Cell Controls--See Figure 1.

\*\*\*\*\* IMPORTANT \*\*\*\*\*  
 NEVER TURN TEMPERATURE REGULATION ON UNLESS WATER TANK CONTAINS WATER  
 \*\*\*\*\*

3.1.1 Figure 1. Description

- A. Plastic lid for 100 ml beaker. Large hole is for placement of electrode. The smaller hole can be used for a thermometer (Not Supplied).
- B. Four-way distribution valve. This is so that up to four different calibration gases can be permanently connected to the calibration cell.
- C. Glass fritted bubbler. This causes dispersion of calibrating gas for rapid equilibrium with fluid.
- D. Glass 100 ml beaker. Fill to about 60 - 80 ml of saline or other appropriate fluid for electrode calibration.

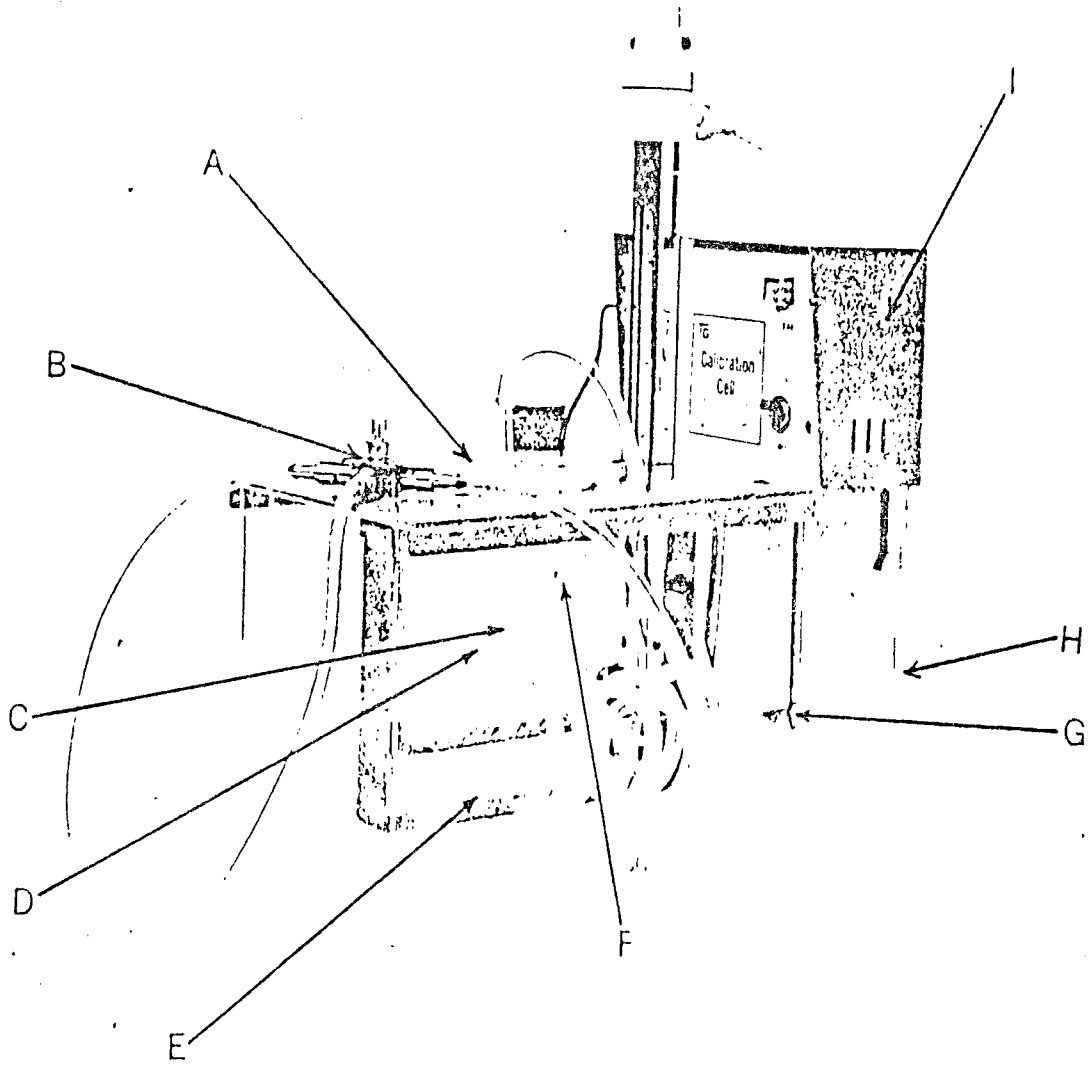


Figure 1. Calibration Cell Model 1251.

- E. Tygon tubing coil for calibrating gas flow through coil and is pre-heated before entering 100 ml beaker.
- F. Reference electrode. This is a sintered Ag/AgCl pellet type reference electrode for stable calibration.
- G. Mechanical Stirrer. This assures uniform temperature in water tank.
- H. Water tank. It is capable of holding 1 gallon of fluid.
- I. Temperature regulator. This regulates the temperature of the water tank.

### 3.2 Initial Operation

- 3.2.1 Fill the water tank with water up to 1/2 inch of tip rim.
- 3.2.2 Fill 100 ml glass beaker with saline
- 3.2.3 Plug the line cord into a three prong 115 VAC receptacle. If a properly grounded three-pronged receptacle is not available, a grounded line adapter should be employed.
- 3.2.4 Turn temperature regulator ON. UNDER NO CIRCUMSTANCE SHOULD THE POWER BE ACTIVATED WITH THE HEATER NOT COVERED WITH FLUID.
- 3.2.5 Position the electrode holder so that microelectrode tip can be suspended in solution.
- 3.2.6 Solution of the beaker may be gas saturated by connecting to a low pressure source (less than 3 psi) to the gas dispersion bubbler.

## IV. TROUBLE SHOOTING

## 4.1 Problems

- 4.1.1 A. Pilot will not light.
  - B. A.C. power inactive
- 4.1.2 A. Erratic electrode response
  - B. Bubbles on electrode tip. Reduce amount of bubbling
- 4.1.3 A. No current through electrode.
  - B. Reference electrode not connected to meter.

## V. REPLACEMENT PARTS

Part Number	Description
5630	Thumb screw 6-32 for clamp
56148	Thumb screw 10-22 for L-bracket.
75153	Hose-lock connector
8601	100 ml beaker
4803	Four-way valve
8602	Gas dispersion bubbler
333	Ag/AgCl reference electrode
4243	L-shaped bracket
42301	Rod clamp.



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- II. Microelectrodes
- III. Applications (Note Specific Use)

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## TRANSIDYNE EQUIPMENT WARRANTY

Transidyne General agrees to correct either by repair or replacement any defect of material or workmanship in the equipment described on the attached card, which develops within one year after delivery of said equipment to the original purchaser, provided that the equipment has been subjected to normal and proper use.

Any component (except where specifically exempted\*), claimed to be defective during the warranty period, should be returned to Transidyne General with transportation charges prepaid. The components will be promptly repaired and returned to the purchaser with return transportation prepaid by Transidyne General. In the event that the component is found not to be defective or found to be damaged by misuse, it will be repaired and returned to the purchaser with repair and transportation billed collect, after notification and approval by the customer.

When requesting service, please contact Transidyne General, or an authorized agent of Transidyne General, for instructions. Under no circumstance should instruments or parts be returned to Transidyne General without prior approval.

This warranty is contingent upon Transidyne General receiving the attached card, and will go into effect upon the installation date of the instrument described on the attached card.

\* Exemptions — Lamps, Heat styluses on recorders

Instrument No. \_\_\_\_\_

Date Installed \_\_\_\_\_

NOTE: The warranty described on the attached card only goes into effect, provided this card is completely filled in and returned to Transidyne General within thirty (30) days after installation of the instrument.

Model No. \_\_\_\_\_ Serial No. \_\_\_\_\_

Date Purchased: \_\_\_\_\_ Seller's Name: \_\_\_\_\_

Date Installed: \_\_\_\_\_

Purchaser's Name (Institution): \_\_\_\_\_

Purchasers Address: \_\_\_\_\_

City \_\_\_\_\_ State \_\_\_\_\_ Zip \_\_\_\_\_

Phone Number: \_\_\_\_\_  
(Area Code)

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