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SUBTITLE: AN INSTRUMENT TO MEASURE THE OXYGEN EQUILIBRIUM CURVE OF BLOOD

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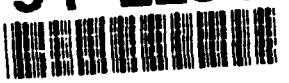

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This was a Phase II SBIR program to design, develop, produce and deliver a standard instrument to measure the oxygen equilibrium curve of blood, and/or hemoglobin solutions. This was done. An instrument was produced that operates under DOS based computer control to carry out the necessary operations and measurements. It uses pumps that we developed to add reagents to solutions under test, electrochemical probes to measure O_2 and CO_2 pressures in solution, maintains constant CO_2 pressure during the test, maintains temperature constant to better than ±0.1°C over the range 15 to 50°C, provides for *in situ* calibration of the electrochemical probes and records and stores the data. The instrument is small and readily portable.

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David W. Blair 6/20/94

PI - Signature Date

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Section 5

Introduction

This is the final report on a Phase II SBIR program to develop an instrument to measure, record and analyze the oxygen equilibrium curve of blood, hemoglobin solutions and/or blood substitutes. The object of Phase I was to design and produce a prototype single instrument package that was an upgraded version of an instrument existing in the Letterman Army Institute of Research to implement existing methods of measuring, recording and analyzing the oxygen equilibrium curve of bloods, hemoglobin solutions and/or blood substitutes. That objective was accomplished and reported at the end of Phase I.[1]

The objective of Phase II was to develop and produce a compact, unified, convenient, reliable and cost effective instrument for measuring the equilibrium oxygenation properties of blood, artificial bloods and hemoglobin solutions. The instrument was to be fully automated, from calibration to presentation of final results, yet within the confines of that automation it was to afford the researcher great latitude in the conduct of the experiment and in the choice of conditions. The instrument was to be configured to produce results that belong to the same statistical family as those of at least some previous workers in the field, *i.e.* to not introduce new uncontrolled variables that obscure comparison with earlier work. Finally, the instrument was be compact and well suited to convenient transport among laboratories, even those in remote locations.

The instrument that was developed meets the above objectives. It can not only advance the pace of research in blood oxygenation chemistry, it may also be adapted to other work on blood chemistry, including work that may be of diagnostic value. It has the capability of adding up to two reagents to a stirred thermostatted cuvette under controlled conditions and monitoring signals from two electrochemical probes that are monitoring the response of the system to those additions. The software is capable of completely running and controlling the experiments. It records all variables, dependent and independent, as functions of time and stores them in standard text format on the hard drive of the MS DOS based computer that is being used. These data include all data (typically temperature, O_2 pressure, CO_2 pressure and cumulative quantities of reagents that are added) from the time the test solution is added to the reactor until the end of the test. This includes any preconditioning that occurs prior to starting the test. As currently configured it can run tests under tightly controlled temperatures from 15 to 50°C. These limits may be extended by

changing gains in the electronics, as will be described in a later section. The instrument that has been developed is quite versatile and it is not restricted to a narrow range of experiments.

Given the above targets, it was important that the action of the instrument on blood duplicate that of earlier work quite closely. This required that the reactor cuvette itself be as inert as possible to the blood that it contains. We chose fire polished quartz as a reactor material because of its well known chemical inertness and minimal surface area caused by roughness. This was a difficult design to fabricate, given the geometric requirements imposed by the instrument, but it was achieved.

Another serious problem that was addressed was getting accurate data given the slow time response of available small electrochemical probes. When a test is started, the instrument measures the initial temperature (which is held constant during the test), O_2 pressure and CO_2 pressure of the blood. It then injects a predetermined quantity of H_2O_2 solution as the first step in oxygenating the blood. It then waits 30 s for mixing to occur and for the probe to reach steady state. It then reads the CO_2 pressure. If that pressure exceeds the initial value, it injects a predetermined quantity of NaOH solution. (Both the quantities of H_2O_2 and NaOH can be changed during a test, either maintaining a current ratio or independently.) It waits another 30 s and again measures CO_2 pressure. If that pressure exceeds the initial value, it again injects NaOH. This sequence continues until the CO_2 pressure falls to or below the initial value. The system then records temperature, O_2 and CO_2 pressures and makes another injection of H_2O_2. This process continues to the end of the test. The ability to vary the size of the injections during the test permits both the NaOH injection to be tailored to the amount required to hold CO_2 pressure constant with a single injection and to vary the rate of oxidation during an experiment to permit varying the spacing of data points for the best optimization of total test time and delineation of non linear portions of the oxygenation curve. This method does not require corrections for the probe response time, as does a method that uses a constant injection at a calibrated rate. Also, in a constant volume experiment such as is usually done in this work, (the system can run constant or variable volume) the 30 s pause allows for extensive mixing and the fluid is homogeneous when an injection is made and the fluid that is ejected from the reactor as a result of the injection is representative of that within the reactor immediately prior to the injection. The injections are relatively fast, and little mixing can occur during their durations.

Allowing time for mixing as the above described method does is important. The vigor of mixing is restricted by the necessity of avoiding damage to the blood cells. Even given a pause, the rate of mixing should be maximized consistent with not damaging the cells. The instrument accomplishes this by using a variable speed propeller type stirrer that points downward into the reactor along its axis. A constant direction stirrer sets up a stable vortex in the reactor that impedes the rate of mixing unless speed is excessively high. This instrument uses a reversing stirrer that regularly reverses direction, destroying the vortex and dissipating its energy in turbulence. It gives rapid mixing with moderate stirring speeds.

During a test, the reactant solution is shielded from the atmosphere by liquid seals of the reactant solution (for constant volume operation) and by a gas swept chamber over the top of the reactor which communicates with it only through small holes that are filled with the liquid seal. The gas in the chamber can be inert or of a composition that matches, say, the CO_2 pressure in the reactant solution. Even with non constant volume operation and no liquid seals, this gives excellent isolation. With constant volume operation and the consequent liquid seals it is even better. O_2 infiltration is not a problem.

The target for temperature control was ±0.1°C. At 37°C we have achieved ±0.002°C. The detector is a thermistor with NBST traceable calibration and it is inserted into the solution adjacent to the stirrer propeller. Control is with a PID algorithm from the computer, and the reactor thermostat is designed to have a high thermal inertia. Heating is from a thermopile that can either input or extract heat from the thermostat, thus operation either above or below ambient operation is possible. A single switch changes the system from heating to cooling. When either heating or cooling is chosen, heat exchange between the thermostat and the surroundings provides the loss that is essential to the control algorithm.

The reactant injections are from 50µl gas tight syringes that are driven by stepper linear actuators. The pulses for the actuators originate in the computer and are output through its printer port to amplifiers that drive the steppers.

Section 6

Body

This section describes the individual components of the system, the software that controls the system, the tests that

were run on the system and the results of those tests. The individual components that are discussed are:

- The thermostatted reactor assembly.
- The reaction cuvette.
- The thermostatted cuvette holder and its base.
- The top section of the cuvette holder.
- The stirrer and associated electronics.
- The thermoelectric heater/refrigerator and its associated electronics.
- The temperature control system.
- The reactant pumps.
- The electrochemical probes.
- The gas control system.
- The computer.
- The electronics
- The software.
- Test results.

Section 6 - 1: The thermostatted reactor assembly.

Figure 6. - 1.1 is a photograph of the thermostatted reactor assembly and figure 6 - 1.2 is an exploded view of the same.

The quartz reaction cuvette fits snugly into the center section of the aluminum heat reservoir (the thermostatted jacket). The well into which it fits is closed at the bottom and open at the top. The interior of the cuvette is a right circular cylinder 0.5 in. diameter by 1.0 in. deep. Four radial holes are located 1/8 in. from the bottom of the cylinder. Two serve as

Figure 6 - 1.1

Photograph of the Thermostatted Reactor Assembly

The stirrer assembly is shown mounted on top of the Reactor assembly. To the rear the two guard tubes for the electrochemical probes protrude to the left and right. The syringe pumps are seen to the left and right foreground with the syringe needles partially inserted into the reactor jacket. In the foreground, a gas line is seen attached to the top of the reactor jacket.

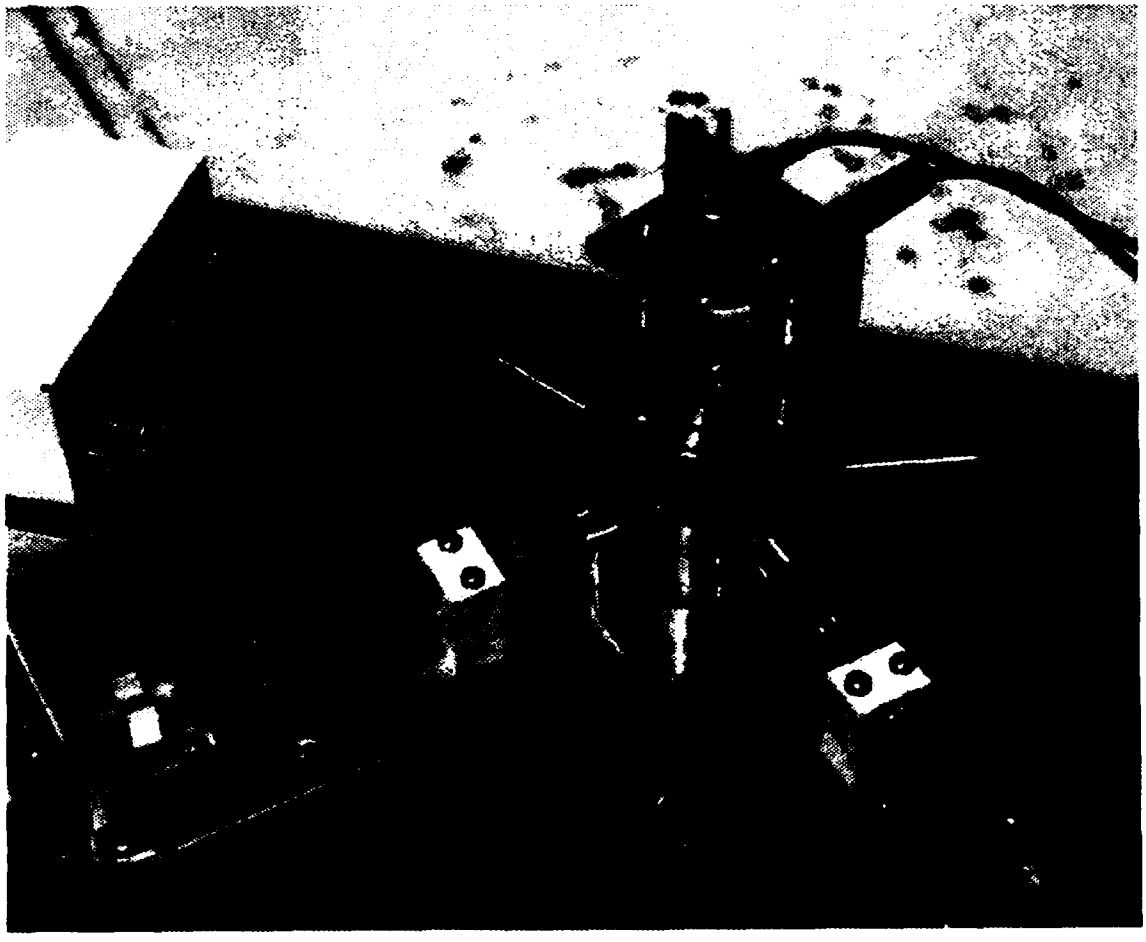
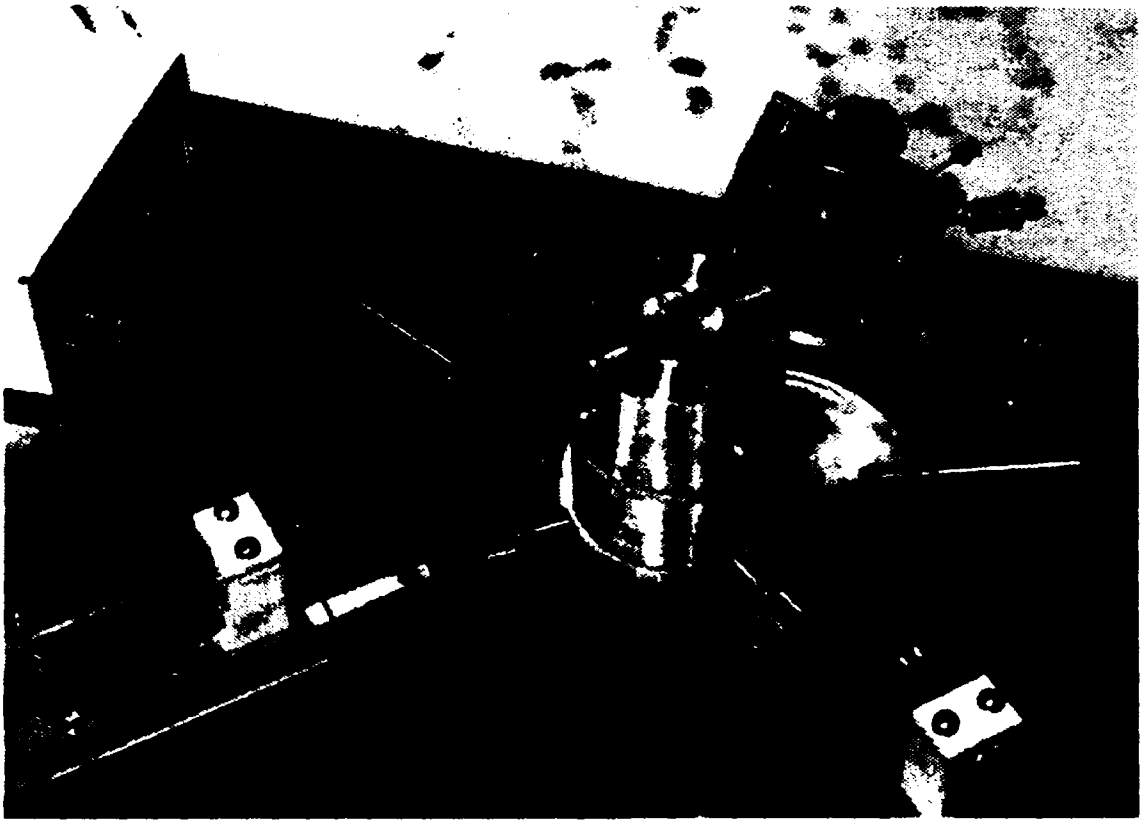


Figure 6 - 1.2

Exploded Photograph of the Thermostatted Reactor Assembly

To the rear is seen the bottom of the top section with its bottom plate in place. The plug on this plate fits into the reactor to define its volume and to form a liquid seal for overflow. The inner "O"-ring forms a radial seal around the reactor. The outer ring is on the back of the transparent plate and forms a seal against leakage from the atmosphere into the sweep chamber in the top section.



ports of entry for syringe needles that send reagents into the reactor and two serve as ports of entry for the electrochemical probes. The locations of the syringes and probes can be seen in figure 6 - 1.1. The aluminum heat reservoir is closed at the top with an annular aluminum ring that is itself surrounded by an annular stainless steel ring. Between the two rings are two gas passages that conduct purge and sweep gas to their destinations. Purge gas is directed downwards into the reactor volume and sweep gas sweeps a separate blanket chamber over the top of the reactor. When this top assembly is connected to the heat reservoir, it becomes part of the reservoir, and the gas that passes through it is conditioned to reservoir temperature before it enters either the reactor or the sweep chamber. A stirrer is mounted over the top and its shaft extends down through the sweep chamber into the reactor. A thermistor extends into the reactor and a hypodermic needle fits into a hole in the reactor cover.

The heat reservoir is attached to a base from which it is thermally insulated, except where a flat square thermopile is lightly pressed with heat transfer grease to give a good thermal; contact. This three part assembly, base, center section and top comprise a single large thermal reservoir. The lower surface of the base is attached to the baseplate of the instrument which serves as a heat source or sink depending upon the action of the thermopile. By simply throwing a switch the thermopile can be changed from a heat pump from the baseplate to the thermal reservoir to a heat pump from the thermal reservoir to the baseplate, *i. e.* from a heater of the reservoir to a cooler of the reservoir.

Section 6 - 2: The reaction cuvette.

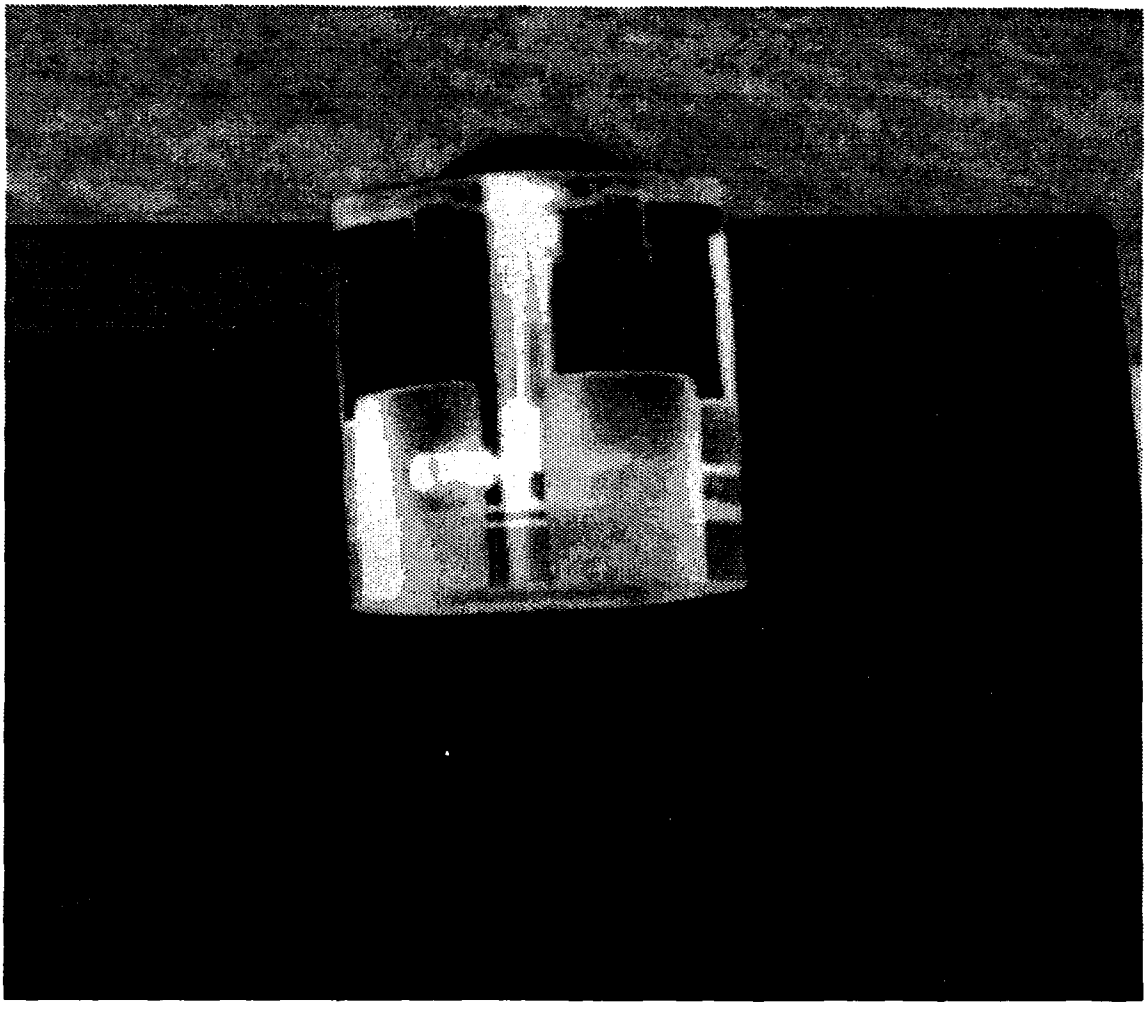
Figure 6 - 2.1 is a photograph of the reaction cuvette. The cuvette is machined from quartz. It was made for us by Precision Glass Products Company of Orland, Pennsylvania. Quartz was chosen because of its great chemical inertness. (It is often used in blood work because of this property). The inside of the cuvette is flame polished to minimize its surface area, and thus its interaction with the reactants.

The syringe needles are sealed to the cuvette by septa that are pressed against the flats at their points of entry. The probes are sealed to the cuvette by "O"-rings (# 2-006) that are sealed against the flats at their points of entry. The probes protrude approximately 1 mm beyond the inner wall of the cuvette, while the needles are inserted about three quarters of the way across the diameter of the chamber. The needles and probes are

Figure 6 - 2.1

Photograph of the Quartz Reaction Cuvette Seen From The Side at an Angle

At the bottom are seen two flats. The one to the left has the entry port for an electrochemical probe, while the one to the right has the entry port for a syringe needle. There is one more of each to the rear. Probe ports are sealed with "O"-rings while needle ports are sealed with septa.



located about 1/8 in. below the propeller of the stirrer.

The cuvette is made in two parts, the upper cylindrical part and a lower base plate 1/4 in. thick. They are joined by a sintered glass joint that gives a hair thin seam at the connection.

Section 6 - 3: The thermostatted cuvette holder.

Figure 6 - 3.1 is a photograph of the cuvette holder seen from the top, and figure 6 - 3.2 is a photograph seen in elevation. In both views, the base is attached to the holder. The bottom of the holder sits on a thermopile which is surrounded by a foam insulator. This combination sits on the base and is held away from it by four plastic standoffs to avoid crushing it. The base and holder are bolted together. The entire assembly is bolted to the 3/16 in. thick base plate, which provides for abundant heat exchange with the laboratory atmosphere.

Section 6 - 4: The top section of the cuvette holder.

Figures 6 - 4.1, 4.2 and 4.3 are photographs of the top section of the cuvette holder seen from the top, bottom and in elevation respectively. Figure 6 - 4.4 is a photograph of the top seen in elevation with its top and bottom plates in place and with the stirrer attached. The stirrer shaft is located on the axis, the thermistor is to the left and the loading needle is to the right. Figure 6 - 4.5 shows a side view of the bottom plate. The 1/2 in. diameter plug slips into the reaction cuvette to limit its volume and provide for constant volume operation. It sets the cuvette volume (calculated) to 1.84 ml with all probes in place. It contains an overflow chamber to contain fluid from overfilling and that which is displaced by added reactants during the test. Both top and bottom plates are made of Lucite.

Two 1/8 in. tubes protrude from the side of the top. One admits purge gas to the reactor and the other admits sweep gas to the chamber above the reactor. The particular gas used is selected from a seven port valve (total of six gases) and its destination (purge, sweep or utility with one spare) is chosen from a five port valve. The gases are temperature equilibrated with the top before being passed to the purge or sweep.

Section 6 - 5: The stirrer and its electronics.

The stirrer with its mounting and the reactor top plate are shown in figure 6 - 5.1 and it is shown mounted on the heat reservoir top plate in figure 6 - 4.4. In the first figure, the thermistor can be seen attached to the mounting. Stirrer speed

Figure 6 - 3.1

Photograph of the Reaction Cuvette Holder Seen From the Top With the Top Section Removed

The cuvette fits snugly into the central hole and is sealed against leakage by the "O"-ring. To the rear in the hole are seen two ports for syringe needles and their seals. The wires in the right foreground are power leads to the thermoelectric heater/cooler.

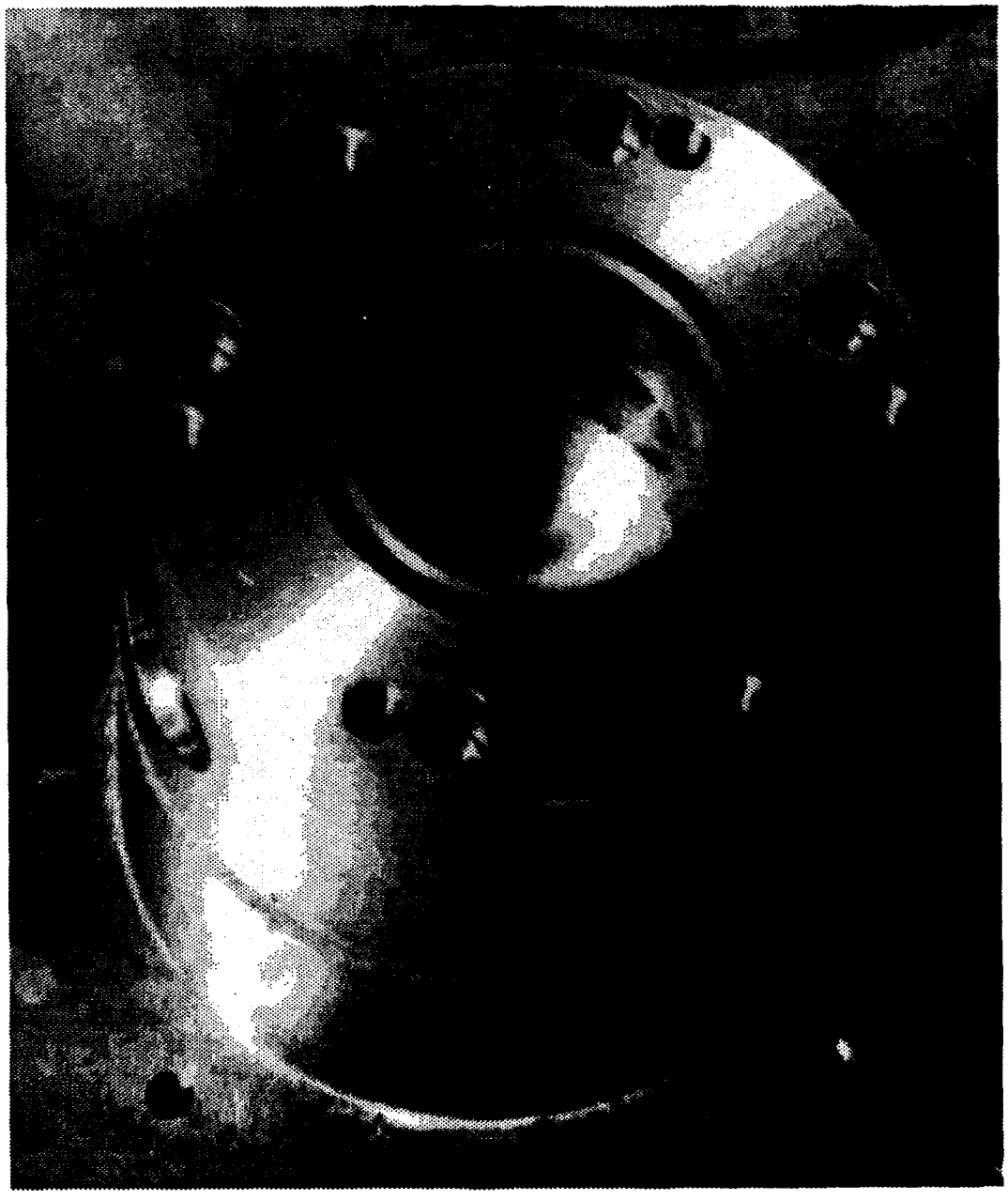


Figure 6 - 3.2

Photograph of the Reaction Cuvette Holder Seen in Elevation

The top section holds the cuvette. At its bottom is seen the edge of the thermal insulator that surrounds the thermoelectric heater/cooler. The section below the insulator conducts heat between the top section and the baseplate of the instrument. The power leads for the thermoelectric device are seen in the foreground as is an entry port for an electrochemical probe assembly.

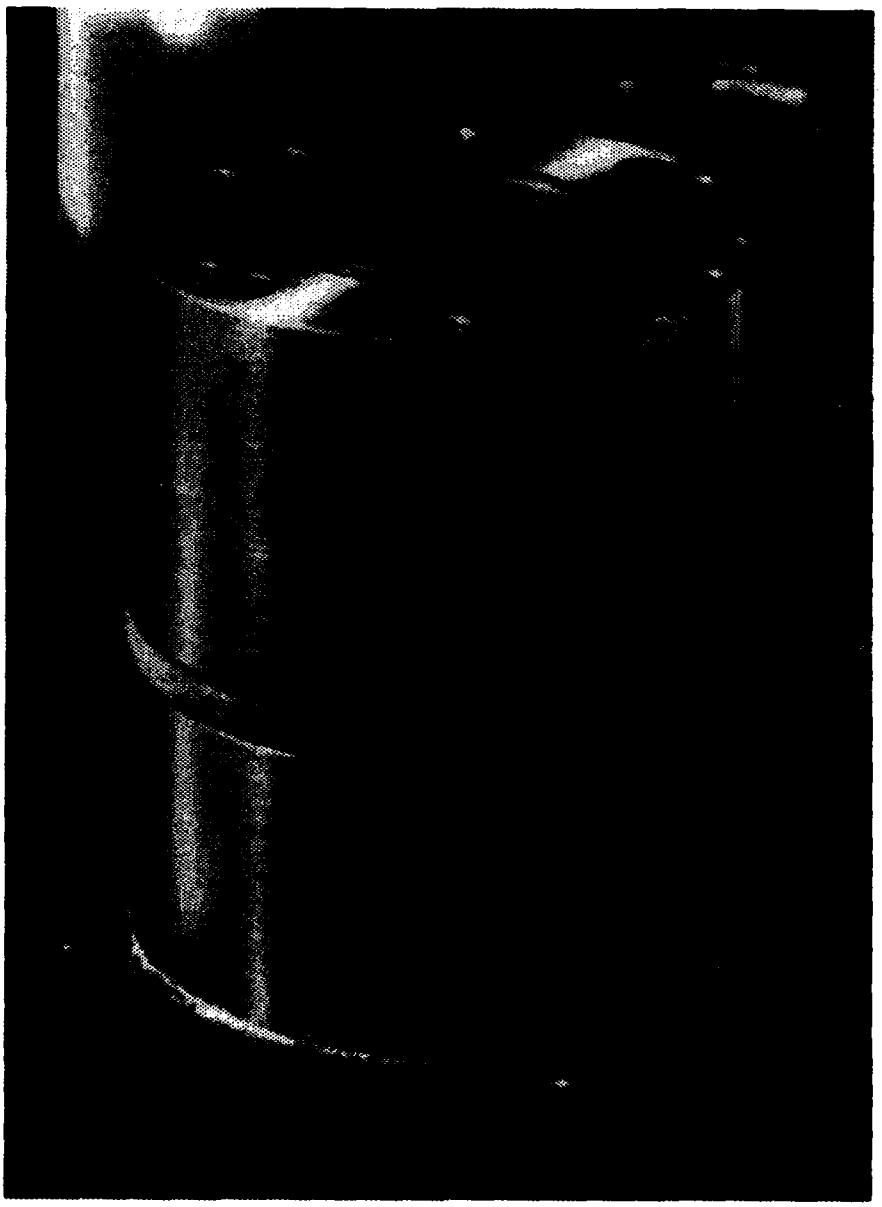


Figure 6 - 4.1

Photograph of the Top Section of the Cuvette Holder Seen From the Top

The "O"-ring seals the top plate of the sweep chamber from the atmosphere. One of the two gas connections conveys gas into the sweep chamber, while the other conveys it down into the reaction cuvette. The central hole is the sweep chamber.

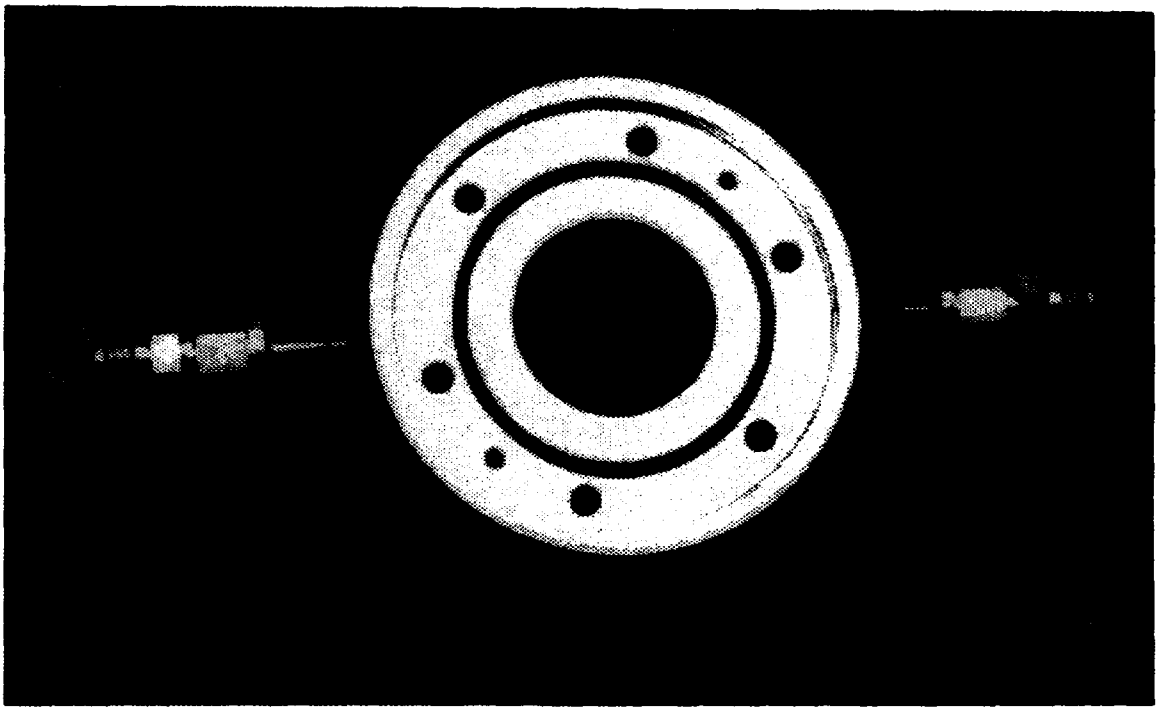


Figure 6 - 4.2

Photograph of the Top Section of the Cuvette Holder Seen From the Bottom

The large outer "O"-ring seals the reaction cuvette against the atmosphere. The cuvette top plate seats against it. The small inner "O"-ring seals the small port that directs gas into the reaction cuvette. The gas connections are seen to the left and right.

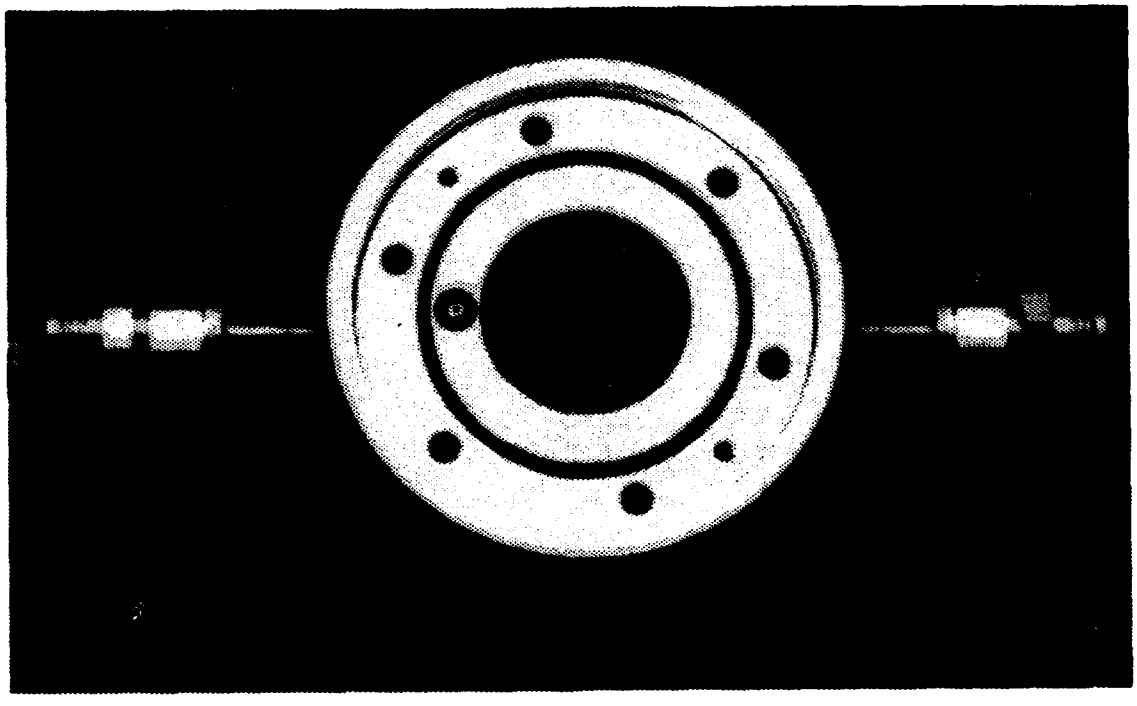


Figure 6 - 4.3

Photograph of the Top Section of the Cuvette Holder Seen From the Side

A scale with one inch markings is shown for comparison.

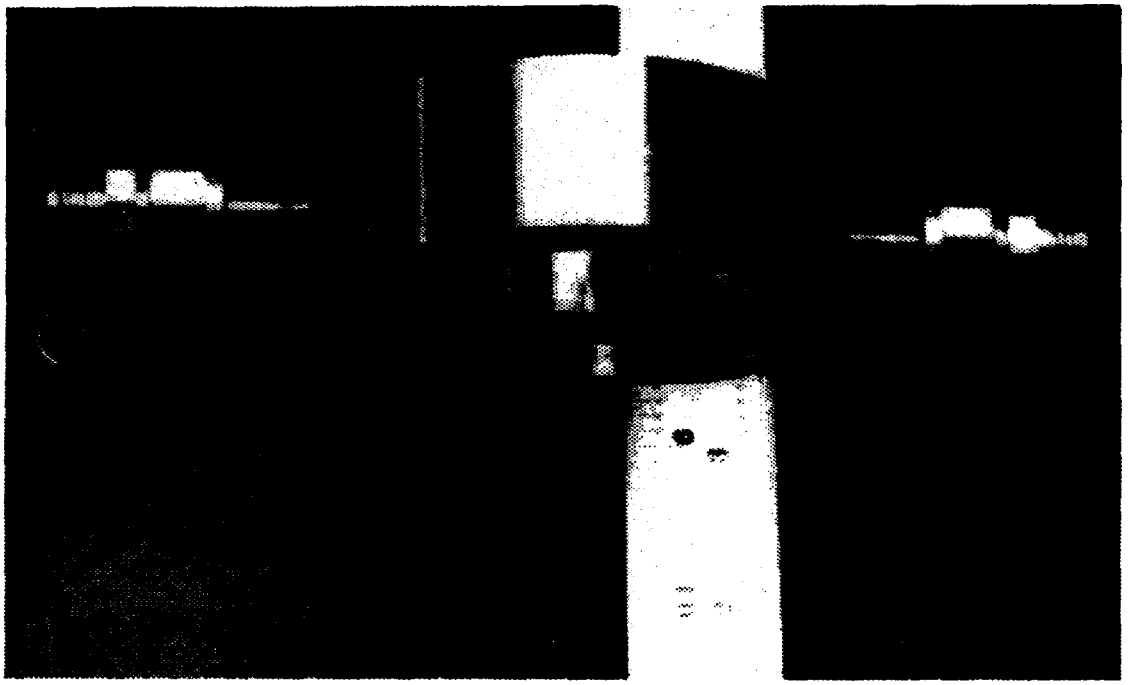


Figure 6 - 4.4

Photograph of the Top Section of the Reaction Cuvette Holder With the Stirrer Mounted and the Top and Bottom Plates in Place

The stirrer propeller can be seen protruding downward in the bottom center with the thermistor immediately to its left. The filler syringe needle protrudes from the top and is bent to the right.

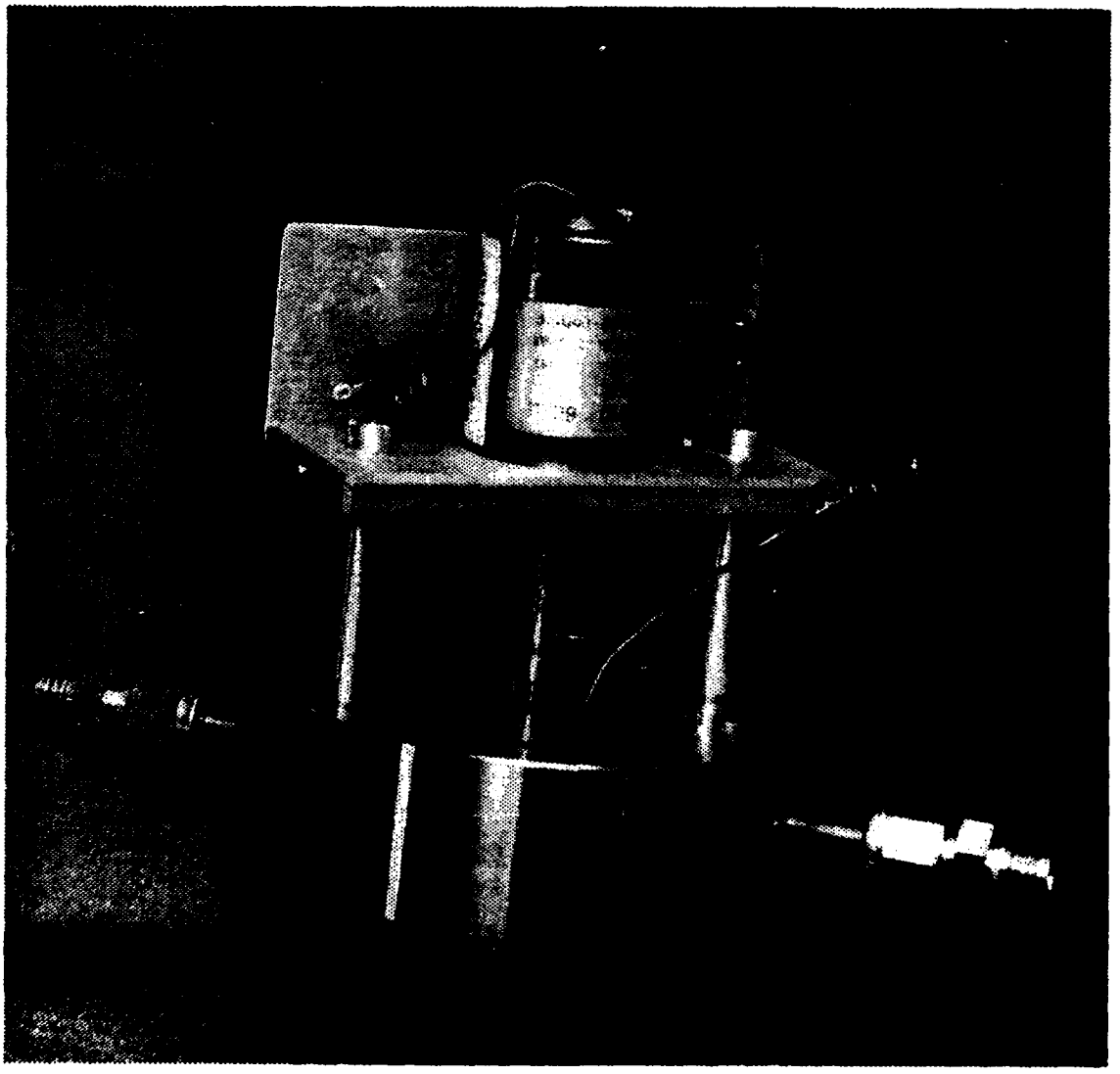


Figure 6 - 4.5

Side View of the Bottom Plate That Fits Onto the Top Section and Plugs The Cuvette Reactor

The small plug on the bottom fits into the reactor. The channel for the stirrer shaft can be seen in it connecting to the bottom of the overfill chamber that forms the liquid seal. The large cylinder on the top extends upwards into the sweep chamber. To the left of it can be seen the fill channel, to the right can be seen a channel that vents the reactor into the sweep chamber through a liquid seal. Protruding from the top is the bushing for the stirrer shaft.

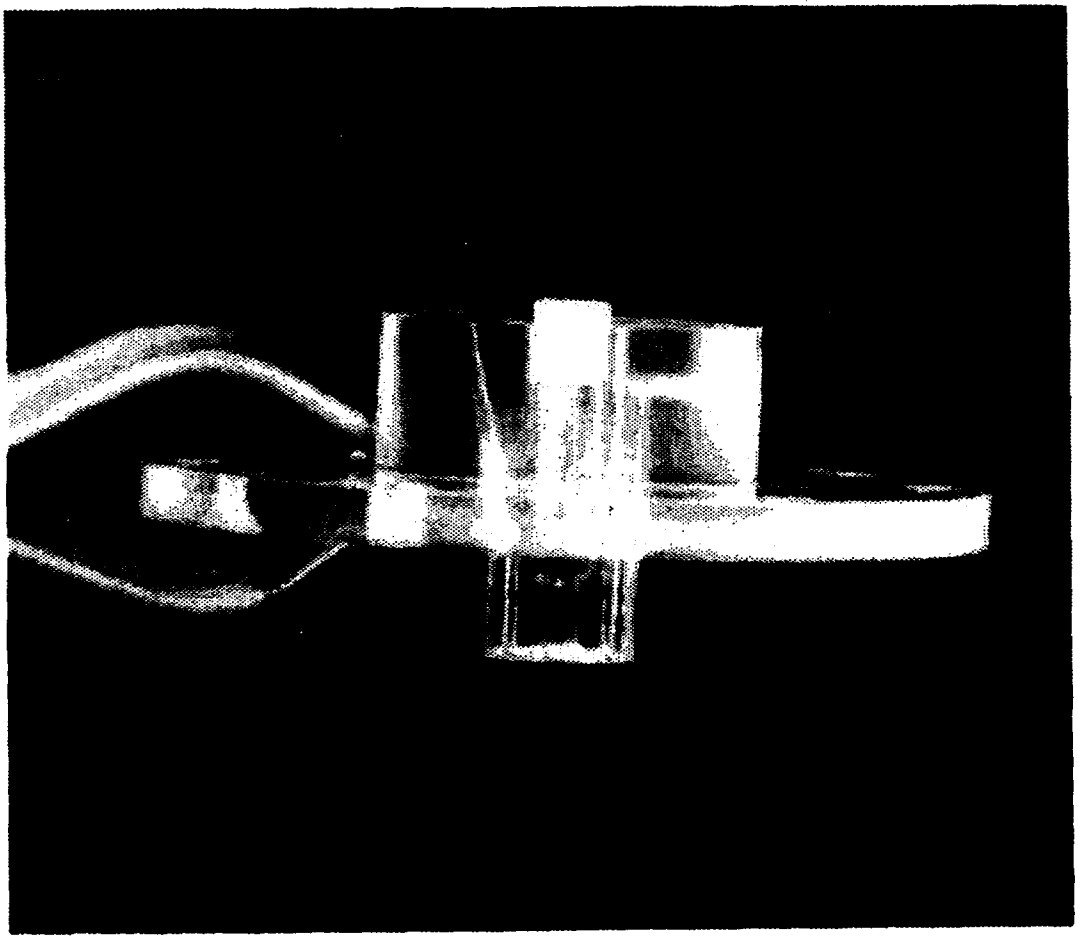
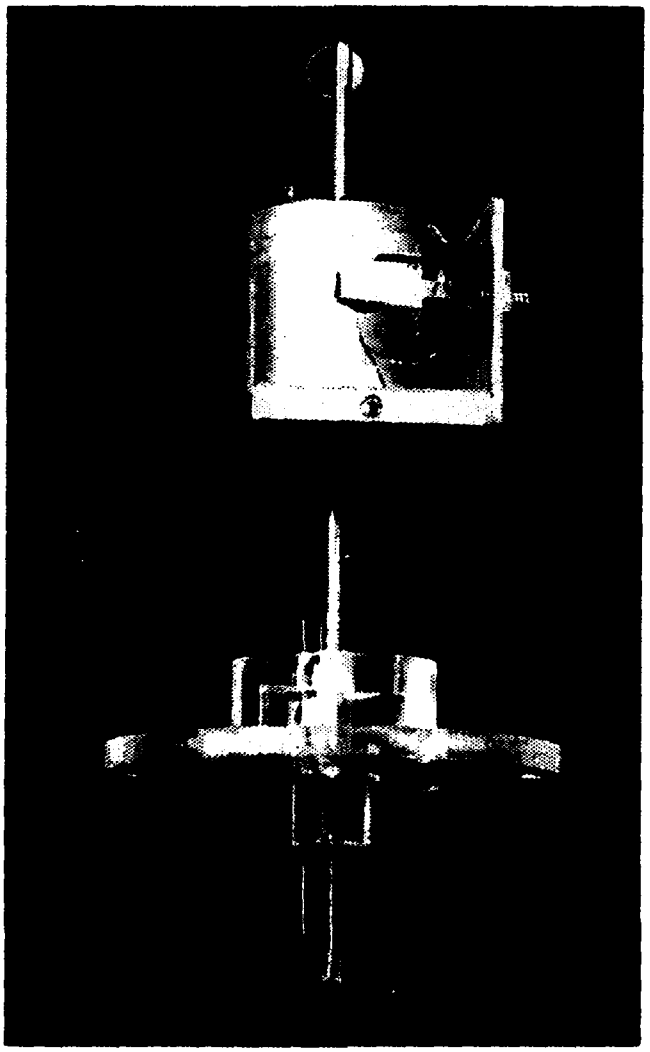


Figure 6 - 5.1

The Stirrer and Its Mounting With the Reactor Top Plate in Place but Without the Top Section of the Reactor Holder

The stirrer motor is seen at the top behind an electronic plug. The stirrer shaft with its propeller attached protrudes down through the top plate reactor plug as does the thermistor immediately to its left.



may be set with the system electronics. It rotates in one direction for ninety cycles of the 60 Hz line. It then stops for ten cycles and then reverses for ninety cycles. This cycle is repeated as long as the stirrer is turned on. It continually sets up an axial vortex in the reactor and then destroys it, creating turbulence in the process. Initially we did not use a reversing system. We found that without reversing the stable vortex led to very slow mixing in the system. A reagent injection would cause an initial pulse from the probes, then no effect for an extended period (as much as a minute) followed by an expected response. Presumably what was happening was that the initial injection swept over the probes and was then entrained in a streamline out of their plane. The vortex being highly stable, the reagent remained on that streamline while it slowly diffused away from it. The diffusion process was slow. This situation could be remedied by much more rapid stirring, but only at the danger of damaging cells in a fluid such as blood. By reversing the motion, mild stirring breaks up the vortex into turbulence which causes rapid mixing.

Section 6 - 6: The thermoelectric heater/refrigerator.

The original breadboard instrument that we constructed provided for heating only. While it had excellent temperature control, we decided that the usefulness of the instrument could be significantly enhanced if we also provided for sub ambient temperature operation. Accordingly we redesigned the prototype instrument to provide cooling as well as heating. The arrangement was described in section 6 - 1.

The thermoelectric unit (thermopile) is a Melcor model CP1.4-127-10L, supplied by Melcor, Materials Electronic Corporation, 1040 Spruce Street, Trenton, New Jersey 08648. It is a square 1.56 in. on a side by 0.19 in. thick. It is a low current, moderate capacity module especially suited for 12 v d.c. operation. It has ceramic insulation, and it contains 127 thermocouples, each 0.10 in. long and 1.4 mm square cross section of a quaternary alloy of bismuth, tellurium, selenium and antimony with dopants. Its operating temperature range is -150 to +80°C. With a hot face temperature of 27°C, a cold face temperature of 17°C, 3.6 a and 14.5 v, this unit will pump 29 w from the cold face and deliver 81 w to the hot face.

The module requires a d.c. voltage for its operation. We use a pulsating d.c. voltage and alter its duty cycle to control the amount of heating or cooling. The heating or cooling is controlled by a d. c. signal in the range -5 to +5 v, with -5 v calling for maximum power, +5 v calling for off and 0 v calling

for approximately half power. The control signal is output from the computer as will be described in a subsequent section.

Under the control system that is used, this unit provides precise control of temperature in the range 15 to 45°C. The limits of the range produce control signal voltages that saturate the D/A output modules that pass the signal from the computer to the power electronics. The range can be extended, but with some loss of sensitivity, by changing the gain in our control circuit electronics.

Section 6 - 7: The temperature control system.

The target precision for temperature control was ±0.1°C at 37°C. We have achieved ±0.002°C. To achieve this precision and to achieve comparable accuracy, we have used custom precision calibrated thermistors obtained from Thermometrics, 808 U. S. Highway 1, Edison, New Jersey 08817. We purchased 6 thermistors, labeled 11 through 16. They were given custom part number A232P-BR14KA153F-CC5HA. They had 0.014 nominal diameter glass thermobeads of nominal 15 kΩ ± 1% at 25°C encapsulated a 0.036 in. maximum diameter by 2-3/4 in. long closed end 20TW, stainless steel tube. The leads are 18 in. long, 38 ga. nickel alloy, bifilar with heavy isomid insulation. They have strain relief sleeves where they exit the tube. They were given schedule 3 calibrations at 15, 25, 37, 50 and 55°C. Thermometric's schedule 3 calibration specifies a temperature accuracy of ±0.01°C over the temperature range 0 to 60°C. The calibration results are shown in Appendix A. They are fitted to two regressions, one gives resistance as a function of temperature, and the other gives temperature as a function of resistance. (In those regressions as reported, Rt refers to resistance at the temperature t, **not** to resistance R times temperature t, *i. e.* to what would more conventionally be written R_t.)

The thermistor tip is located just above the propeller of the stirrer. The thermistor then extends upward through the reactor, through the liquid seal, through the sweep chamber, through the top plate and into the atmosphere. The sweep chamber is thermostatted and within and above it the thermistor is thermally insulated. The thermistor projects approximately 1 1/8 in. into the atmosphere. The combination of a fine probe with its sensitive tip in a stirred liquid and a long (approximately 2 in.) path through a thermostatted region with thermal insulation where it is not in liquid gives little opportunity for temperature error related to heat transfer along the thermistor.

The thermistor can be interfaced to the A/D converter that feeds temperature information to the computer or to a digital voltmeter through either of two circuits. The one that is used for control by the software is a bridge circuit that measures the thermistor resistance and passes a voltage proportional to that resistance directly to the A/D interface. That voltage multiplied by 25000 gives the resistance of the thermistor. That multiplication is done by the software, and the result is put into the regression formula for the particular thermistor to produce the equivalent temperature. That temperature is reported and used for control.

An alternative circuit that is not used for control uses a bridge circuit that is designed to operate over the range from 25 to 50°C. The bridge signal is conditioned in the circuit to output a voltage that is directly proportional to the temperature. It is 250 mv at 25°C and 500 mv at 50°C. Its accuracy is about 1% within that range. (The six thermistors match within 1% so any of them may be used with this circuit.) This circuit is handy for reading the temperature directly on a digital voltmeter during various operations where greater accuracy and automatic control are not required.

The signals from the thermistor are sent to the computer through a Metrabyte model M1121 signal conditioning module. (Three of these modules are used in the instrument, one each for thermistor, CO_2 probe and O_2 probe signals. An additional M3131 module is used for signal output to the heater/refrigerator circuitry. These units are all daisy chained together off of a single RS232 port, COM1.) The M1121 A/D input modules accept signals up to 1 v and output to an RS232 port. Their specifications are:

Analog:

- Single channel analog 1v input.
- Maximum CMV, 500 Vrms input to output at 60 Hz.
- 15 bit measurement isolation.
- Leakage current, input to output at 115 Vrms, 60 Hz < 2μA rms.
- 8 conversions per second.
- Autozero.
- Autocalibration.
- No adjustment pots.

Digital:

- 8-bit CMOS microcomputer.
- All scaling, linearization and calibration performed digitally.
- Nonvolatile memory eliminates pots and switches.

Digital Filtering:

- Small and large signal with user selectable time constants from 0 to 16 seconds.

Digital Inputs:

- Open collector to 30 v without damage.
- Switching levels: High, 3.5 v min., Low, 1.0 v max.
- Internal pull up resistors direct switch input.

Digital Outputs:

- Open collector to 30 v, 30 ma max. load.

Communications:

- RS-232, RS-285.
- Up to 124 multidrop modules per host communications port.
- User selectable channel address.
- Selectable baud rates: 300, 600, 1200,2400, 4800, 9600, 19200, 38400.
- ASCII Format command/response protocol.
- Can be used with "dumb" terminal.
- Parity options: odd, even, none.
- All communications setups (address, baud rate, parity) stored in nonvolatile memory.
- Checksum can be added to any command or response.
- Communications distance up to 10,000 feet.

Event Counter:

- Up to 10 million positive transitions at 60 Hz max., filtered for switch debounce.

Power requirements:

- +10v to +30v, 0.75w max.

Environmental:

- Temperature Range: Operating -25 to +70°C.
  Storage -25 to +85°C.
  Relative Humidity: 0 to 95% noncondensing.

The M3131 D/A output module outputs a signal in the range ±5 v. Its specifications are

Analog:

- Single channel analog ±5 v output.
- Input isolation, 500 Vrms
- Resolution, 12 bit.
- Accuracy, 0.1% FS (max) accuracy over temperature.
- Throughput, 1000 conversions/s.
- Settling time to 0.1% FS, 300 µs typ (1 ms max).
- Output slewing
  Manual mode
  (-FS to +FS), 5 s.

Programmable.

- Output slew rate, 0.1 v/s to 10,000 v/s.
- Auto zero and auto calibration, no adjustment pots.
- Voltage compliance, +12 v.
- Output drive, Short circuit current 5 ma min., 10 ma, max.

An M1121 is interrogated by the computer at software controlled intervals for the resistance signal in volts. That signal is converted to resistance by the software (by multiplying by 25000) and the resistance value is converted to temperature by the software using the regression formula for the particular thermistor. The temperature is then sent to the PID control algorithm to generate a control signal. The control algorithm is a relative algorithm, *i. e.* the control signal is a function of the change in the error rather than of the absolute error. This avoids windup problems.

The algorithm that was used was taken from the book by Bucek[2]. It was the discrete implementation of the continuous time-dependent PID control expression[3]:

1. $u(t) = K_p e(t) + K_i \int e(t)\, dt + K_d\, de(t)/dt$

$u(t) \equiv$ the signal to be output at time t.

$K_p \equiv$ the proportional control constant.

$e(t) \equiv$ the error at time t, *i.e.* the set point minus the output.

$K_i \equiv$ the integral control constant.

$K_d \equiv$ the differential control constant.

The discrete implementation of eq.(1) is[4]:

2. $u_k = u_{k-1} + \alpha e_k + \beta e_{k-1} + \gamma e_{k-2}$

where u is the control signal at the k^{th} interval and e is the error at the k^{th} interval.

The parameters α, β and γ are given by[5]:

$$\alpha = (K_p + K_i T/2 + K_d/T)$$

$$\beta = (K_i T/2 - K_p - 2K_d/T)$$

$$\gamma = (K_d/T)$$

where

$T \equiv$ the sampling time interval, s.

Alternatively, if one defines the integral time constant T_{iD} as:

$$T_{iD} \equiv K_p/K_i$$

and the derivative time constant T_{Dd} as:

$$T_{Dd} \equiv K_d/K_p$$

then

$$\alpha = K_p(1 + T/2T_{iD} + T_{Dd}/T)$$

$$\beta = K_p(T/2T_{iD} - 1 - 2T_{Dd}/T)$$

$$\gamma = K_pT_{Dd}/T$$

The code that implements this algorithm is a code with the variables K_p, T, T_{iD} and T_{Dd} being input variables and the α, β and γ being calculated.

Original tuning was performed on the breadboard (heater only) unit. The tuning procedure used the transient response method to determine the initial values of the control constants[6]. The voltage applied to the heater control circuit was 40v. The heaters were four 25w heaters (for 110v input) with two series pairs connected in parallel (to give again 25w with 110v input). The temperature was measured with thermistor # S/N - 2 (from an earlier set of thermistors) immersed in the reactor, which was filled with water and stirred. Thus the tests simulated the thermal control problem of a real blood oxygenation experiment quite well. The step input response gave initial values of:

$K_p = 2.711$

$T_{iD} = 120s$

$T_{Dd} = 30s$

Tuning was started using these values and T = 10 s. (The control circuit gave the heater voltage full on, *i.e.* 40 v, when the control signal was -5 v and full off, save for a very small offset, when the signal voltage was 5.2 v). Numerous tests were run over parameter space varying each of the four parameters K_p,

T, T_{iD} and T_{dd} to find an optimum combination of response time and stability. Our design of the apparatus was then changed to replace the electrical resistance heaters with the above mentioned thermoelectric units, and the system was then retuned to accommodate those units. Numerous tests were then run to again optimize the response time and stability. With the current system, the parameters Kp = 2.0, T = 10, Tid = 240, and Tdd = 75 give excellent control as can be seen from the cold start and restart (with water in the reactor) results shown in figures 6 - 7.1 and 6 - 7.2. These values of the parameters have been set as default values in the software, which permits them to be readily changed for any given experiment.

Section 6 - 8: The Reactant Pumps.

There are two reactant pumps, one for H_2O_2 and one for NaOH. They are syringe pumps of our own design. They are designed to use Hamilton gas tight syringes. As supplied, the software is set for Hamilton model # 1705 syringes. The bore of this syringe is reported by Hamilton to be 0.0406 in. This gives a calculated value of 21.2150 µl delivered per 1 in. stroke. The software is set to require 400 steps per revolution of the 40 pitch drive screw. Thus it requires 754.1833 steps to deliver 1 µl. The value 754.2 is used in the software to calculate the number of steps needed to inject a predetermined quantity of reagent. The resulting value is rounded to the nearest step by the software.

The pumps may also be used with Hamilton model # 1710 syringes (100 µl), which have a 0.05740 in. bore (requiring 377.316 steps/µl with a 40 pitch screw and 400 steps/rev.) or with Hamilton model # 1725 syringes (250 µl), which have a 0.12820 in. bore (requiring 75.640 steps/µl with a 40 pitch screw and 400 steps/rev.).

The screws are 1/4 - 40 rolled threads in cold rolled steel with bearing bronze drive nuts. The motor, syringe holder and guide track are mounted on a track that permits the entire assembly to be moved back and fourth for the purpose of inserting the needle into the reactor and removing it therefrom. A pump is shown in figure 6 - 8.1.

Section 6 - 9: The Electrochemical Probes.

There are two gas sensing electrochemical probes in the system, one for O_2 and one for CO_2. They are both produced by Microelectrodes, Inc. of Londonderry, New Hampshire. The O_2 probe is a model MI-730 Micro-Oxygen Electrode. Its specifications are:

Figure 6 - 7.1

Cold Start

Test File 010594D.dat

Kp = 2.0, T = 10, Tid = 240, Tdd = 75

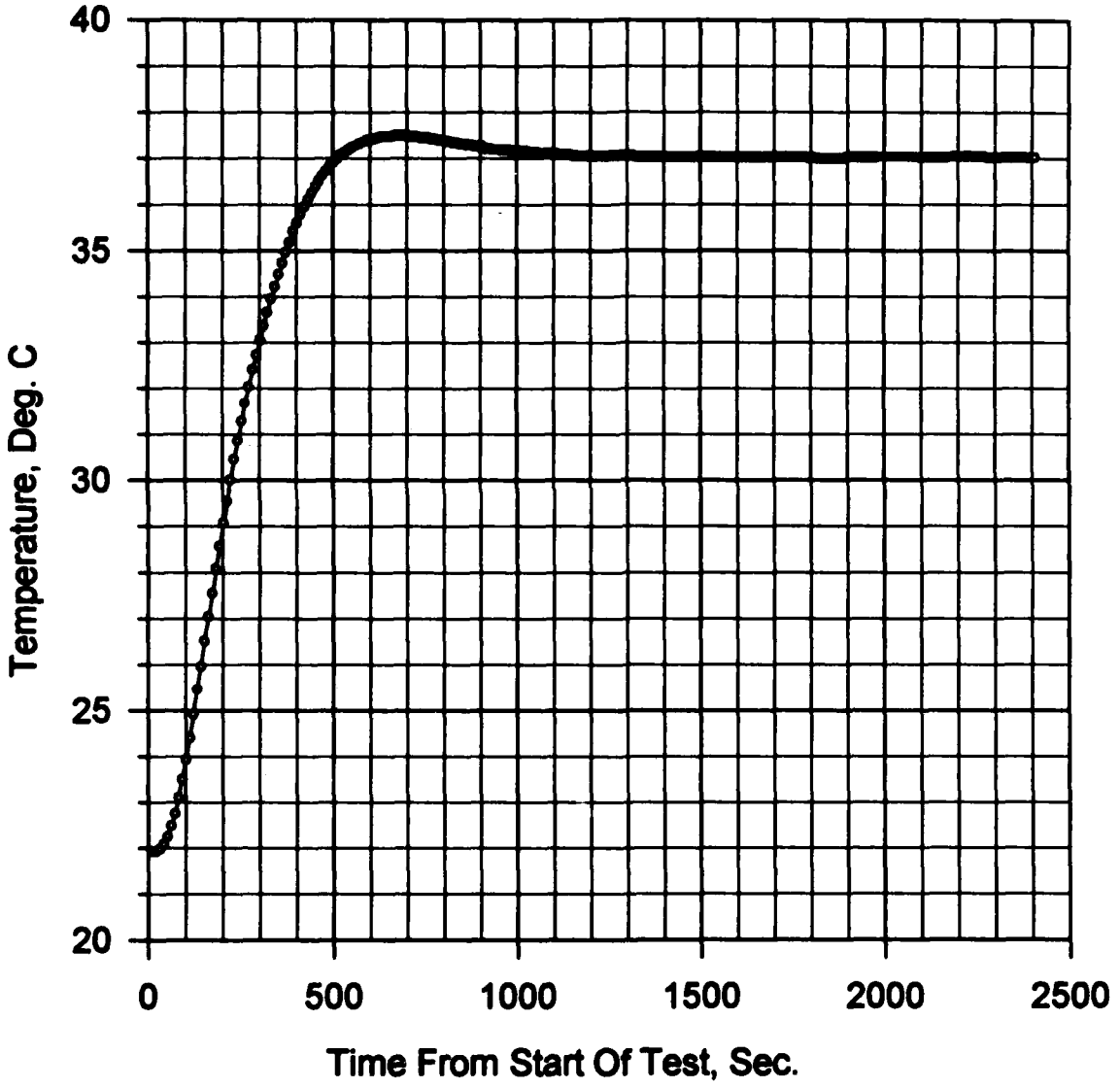
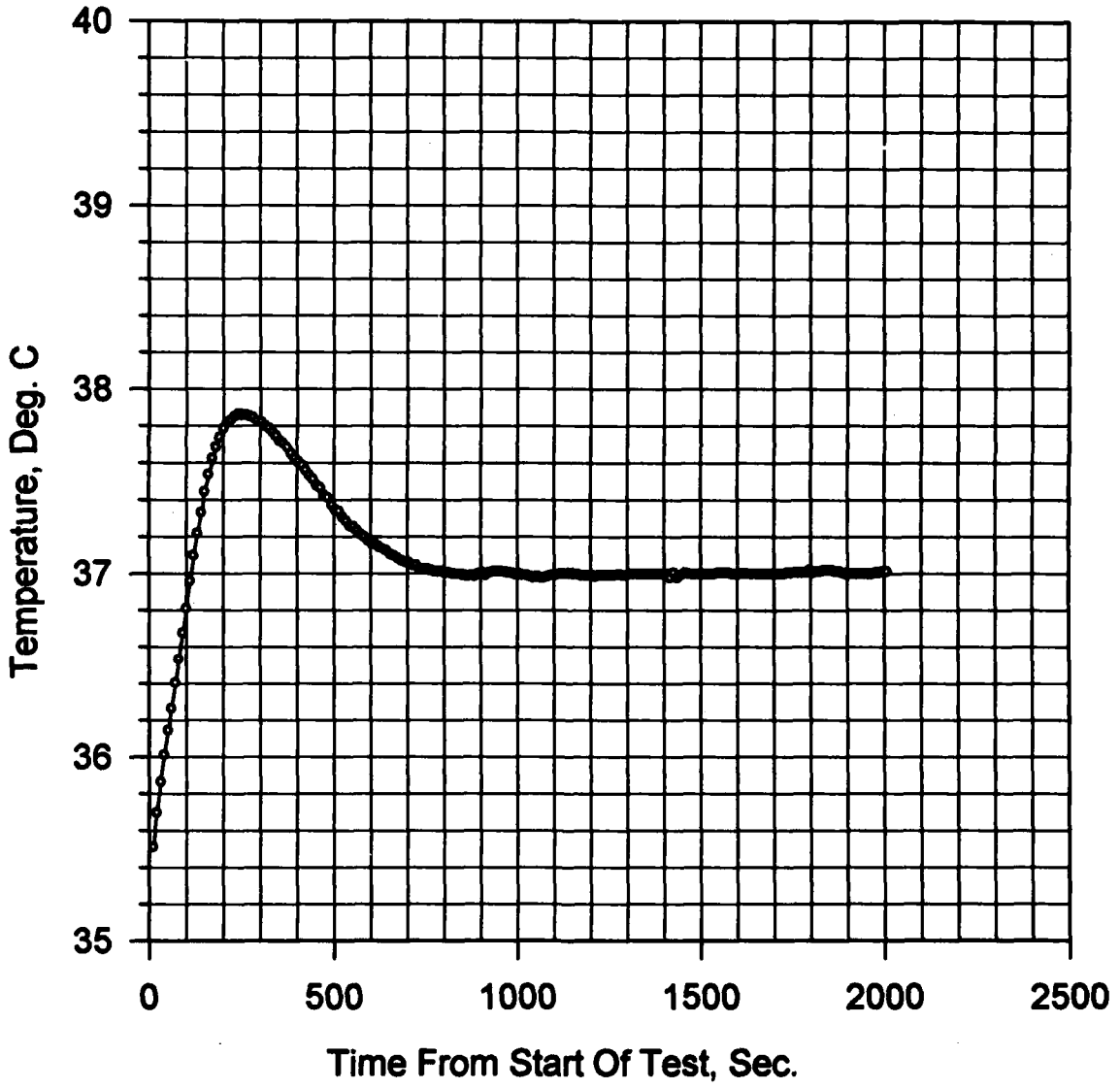


Figure 6 - 7.2

Restart At 35.1 Deg. C

Kp = 2, T = 10, Tid = 240, Tdd = 75

Test File 010594RD



- Total length..... 8.6 cm.
- Lead length (custom)..... 18 in.
- Body (outer diameter)..... 3 mm.
- Tip (outer diameter)..... 3 mm.
- Response range..... 0% to 100%.
- Response time..... less than 20 s.
- Depth of immersion..... 0.1 mm.
- Reference electrode type.... Ag.
- Sensitivity ..... 1700 pA in air at 25°C.
- O_2 consumption in air at 25°C. 2.5×10^{-4} µl/hr.

The probe has replaceable tips which are available in the model MI-730A Replacement Membrane Housing Kit, which includes six membrane replacement tips, one bottle of electrolyte solution and one syringe with filling tips.

The CO_2 probe is a model MI-720 Micro-Carbon Dioxide Electrode. Its specifications are:

- Total length..... 8.6 cm.
- Lead length (custom)..... 18 in.
- Body (outer diameter)..... 3 mm.
- Tip (outer diameter)..... 3 mm.
- Concentration range..... 10^2 to 10^4 M (440 to 4.4 ppm).
- Sensitivity..... 0.4 mv/mm CO_2.
- Interferences..... weak volatile acids.
- Response time..... less than 1 min.
- Depth of immersion..... 0.1 cm.
- Reference electrode type.... Ag-AgCl

This electrode also has replaceable tips. They are available in the model MI-730 Replacement Membrane Housing Kit, which includes six membrane tips, one bottle of electrolyte solution and one syringe with filling tips.

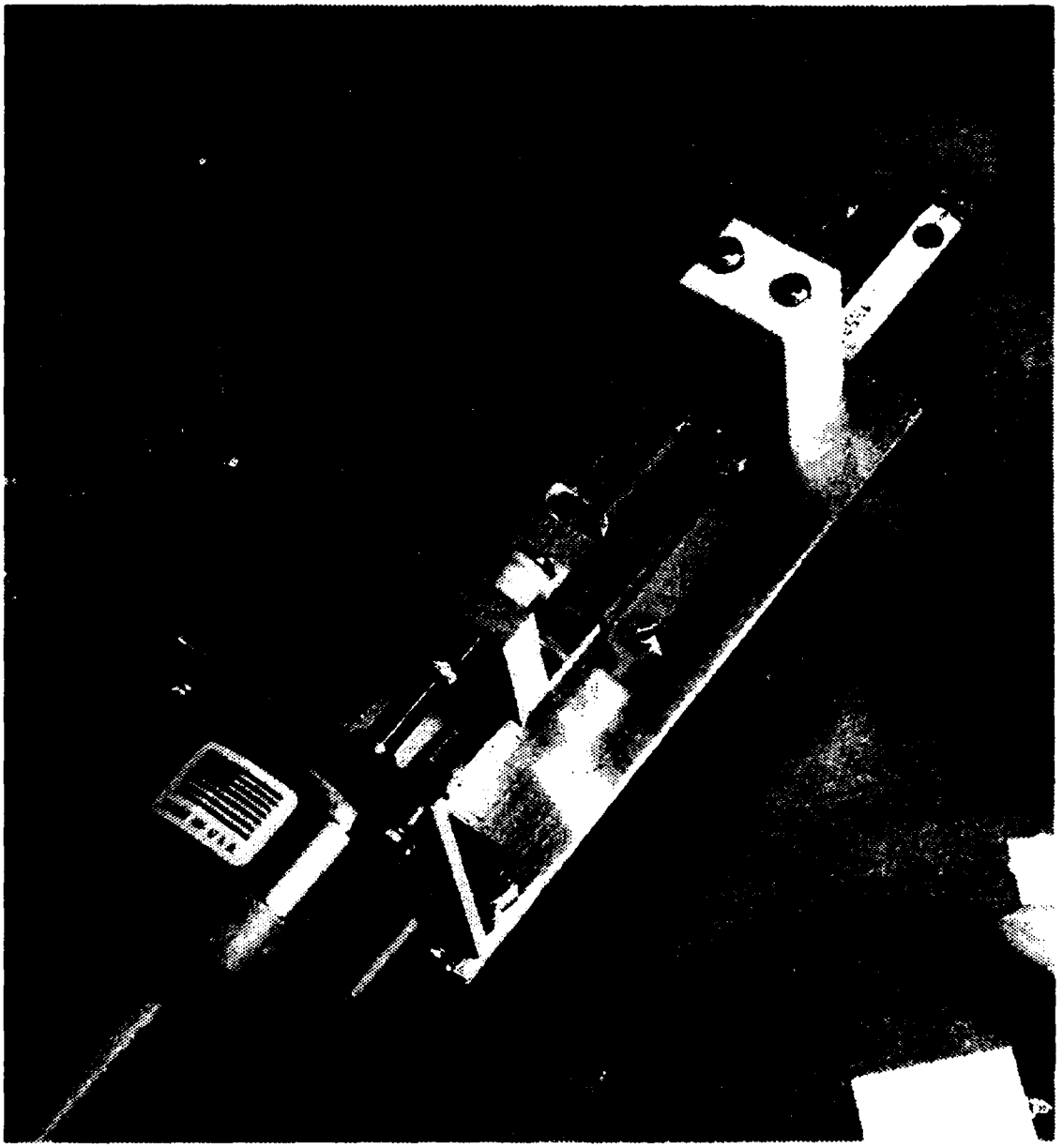
The O_2 electrode is a Clark type electrode. Its output is a current. Our electronics supply the 0.8 v polarization voltage, invert the current signal, amplify the resulting voltage signal and pass it to a Metrabyte M1121 A/D interface which in turn passes it to the computer that is controlling the experiment.

Electronically, the CO_2 electrode is a pH electrode. Its voltage output signal is passed to our electronics (input impedance $>10^{14}$ Ω) which amplify it and pass it to another Metrabyte M1121 A/D interface which then passes it to the computer.

Figure 6 - 8.1

Photograph of One of the Syringe Pumps

The stepper driven linear actuator is shown mounted on the bracket to the left. Its 1/4 - 40 screw has an anti-backlash fitting on the right hand face of the motor housing. The screw is held from turning by the fitting near its right end, and that fitting is mounted on a slide. A fitting on the right end of the screw holds the plunger of the 50µl syringe. The entire assembly is mounted on another slide, shown protruding from below it on the right, that allows it to be moved relative to the reactor. A stepper driver is partially shown to the upper left.



These probes serve their purposes quite satisfactorily aside from their rather long response times, which require adequate periods (≈ 30 s) to settle. This is less burdensome than it might at first seem, because after an injection of reagent a waiting period must be allowed for thorough mixing to occur. Our software supplies the required waiting period: 30 s. An advantage of our system of discrete injections and waiting periods is that all actions that become part of the data occur with a homogeneous reaction system and steady probe signals. The relatively slow response of the probes in part stems from their small size, which is necessary if they are to fit in our reactor. However, this small size leads to a very low gas consumption rate and accompanying error from gas consumed by the detectors but charged to the reaction.

The membranes of both probes are Teflon. The manufacturer claims significantly faster response with silicone membranes (≈ 10s), but these membranes are in precommercialization at this time.

If used regularly, every day or so, the probes are relatively stable. If they are to be stored for any extended period they should be stored in a storage solution that is available from the manufacturer. Long periods of non-use, over one week, usually require that the tips be replaced and refilled.

The electrodes should be recalibrated each day to avoid losing data as a result of changing calibration. Our system provides a convenient and quick calibration procedure as part of the instrument, and it makes calibration fast and simple. It should be used frequently. Calibrations should never bracket more data than the experimenter can afford to lose. That caution is not specific to this instrument, it is good practice for any experimental apparatus.

Section 6 - 10: The gas control system.

The gas control system is constructed to supply any of six gases to any of four destinations. For testing we have used five gases, pure N_2, 5% CO_2 in N_2, 3% CO_2 + 3% O_2 in N_2, 10% CO_2 + 10% O_2 in N_2, and 21% O_2 in N_2. We have provided lines to three destinations: the reaction cuvette cavity, the reaction cuvette sweep gas blanket chamber and a utility line to be used where needed, as in conditioning blood prior to injecting it into the reaction cuvette.

The gases should be provided from regulated sources at about 1 psig. They pass first to the selection valve, then to a variable area flowmeter with a 60 mm scale and a metering valve,

then to the destination valve. When calibrating, the gas is passed into the reaction cuvette (purge position). During an experiment, the gas is passed to the sweep chamber (sweep position).

The purge and sweep paths circle around the top of the thermostat block which brings the gas to the set temperature before it is sent either into the cuvette or the sweep chamber.

Under normal operation, the flow is set to 30 on the scale of the flowmeter. Provision for humidification must be made separately.

Section 6 - 11: The Computer.

The system requires an MS DOS based computer with at least one parallel printer port and one RS-232 serial port for its operation. We used a Toshiba model 4400C notebook computer. It has an Intel DX2/486 microprocessor and a color monitor, which is necessary to operate with our software. Otherwise the computer represents considerable excess capability, but it has the advantage that it is adequate for all subsequent operations that one might wish to perform with the data. To run the experiment, a much slower unit would be adequate, and even a faster unit would be less expensive in a desktop version. The choice of computer is largely a matter of what the user wants in capability for this very generally useful item.

Section 6 - 12: The Electronics

While the original breadboard instrument used resistance heaters controlled by *via* a triac directly from the ac supply, the revised unit uses a thermoelectric device operating from a dc supply. This has the advantages that, because of the geometries involved, thermal coupling to the thermostat block is improved and the thermoelectric unit can provide either heating or cooling, which can be chosen merely by throwing a switch. The prototype, unit comprises only two packages, the mainframe instrument and an electronics control box, together with a personal computer to control the total operation. The electronics control the system, drive the thermoelectric heater/cooler and the stirrer, and read the two electrochemical probes and the thermistor. They provide A/D and D/A interfacing and an alternative of either manual or computer control. They also provide direct readout of signals from the electrochemical probes and thermistor and direct temperature readout (through analog circuitry) of the temperature. The direct temperature readout does not include the specific calibration for each thermistor as does the computer readout, but the thermistor

calibrations are within about one percent of each other, so it is a useful adjunct to the computerized data acquisition.

A block diagram of the system is shown in figure 6 - 12.1. The main instrument carries the thermostatted block and reaction chamber, the reagent pumps with their stepper motors and driving electronics, the gas distribution system, the stirrer, the thermoelectric heater/cooler, the thermistor for thermal control and data acquisition, the electrochemical probes and a dual preamplifier for the probes.

The mainframe instrument is connected to its associated computer (which can be any MS DOS based computer) by a cable which plugs into the parallel printer port (Lpt 1) of the computer. This cable carries control pulses from the computer to the stepper motor drivers. Five other cables connect the mainframe to the electronics box. They are the stirrer cable, the thermistor cable, a power cable for the stepper drivers and thermoelectric heater/cooler, a power cable for the dual preamp and a signal cable carrying signals from the two electrochemical probe preamps.

The electronics box contains three power supplies, the temperature control circuit, the stirrer driver electronics and the A/D and D/A converters which interface the system with the computer. A single cable from the electronics box carries the signals between the A/D and D/A converters and the RS-232 (COM1) port of the computer.

The mainframe instrument as seen from above is shown in figure 6 - 12.2 and the front panel of the electronics box is shown in figure 6 - 12.3.

A thermistor located in the reacting fluid in the reaction chamber is used to measure the reaction temperature. It may be interfaced to its A/D interface with the computer by either of two circuit blocks. The temperature converter block is a bridge circuit designed to operate over the range from 25 to 50°C. Its output is a voltage directly proportional to the chamber temperature, being 250 mv at 25°C and ranging up to 500 mv at 50°C. Its accuracy is about one percent in this range. With appropriate software, this circuit could be used to control the temperature, however, we have not provided the necessary software. The temperature may be read in direct mode by switching to the digital voltmeter on the electronics box. This can be done when not operating under computer control. <u>When operating under computer control, and thus using the resistance measuring circuit, do not switch to the direct temperature</u>

Figure 6 - 12.1

Block Diagram of the Blood Oxygenation Instrument

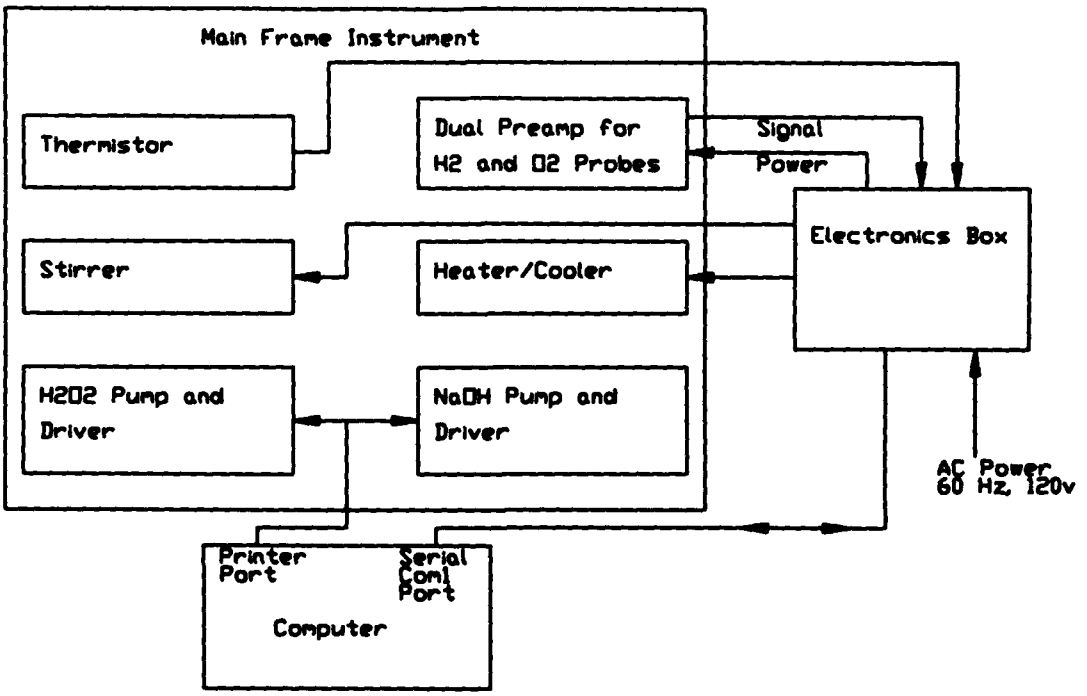


Figure 6 - 12.2

Photograph of the Instrument (Without the Electronics Box) Shown Seen From the Top

The syringe pumps are shown at the top and bottom left with their drivers between them on the left. The reactor assembly is shown in the center right with an electrochemical probe inserted in its sleeve in the foreground. Gas connections are shown to the right and left on the reactor assembly, and the fill needle is seen on the upper left of the assembly. Two small electronic boxes are seen to the rear. The one to the right has the preamps for the electrochemical probes, and the one to the left has the circuits that feed pulses from the computer to the stepper drivers.

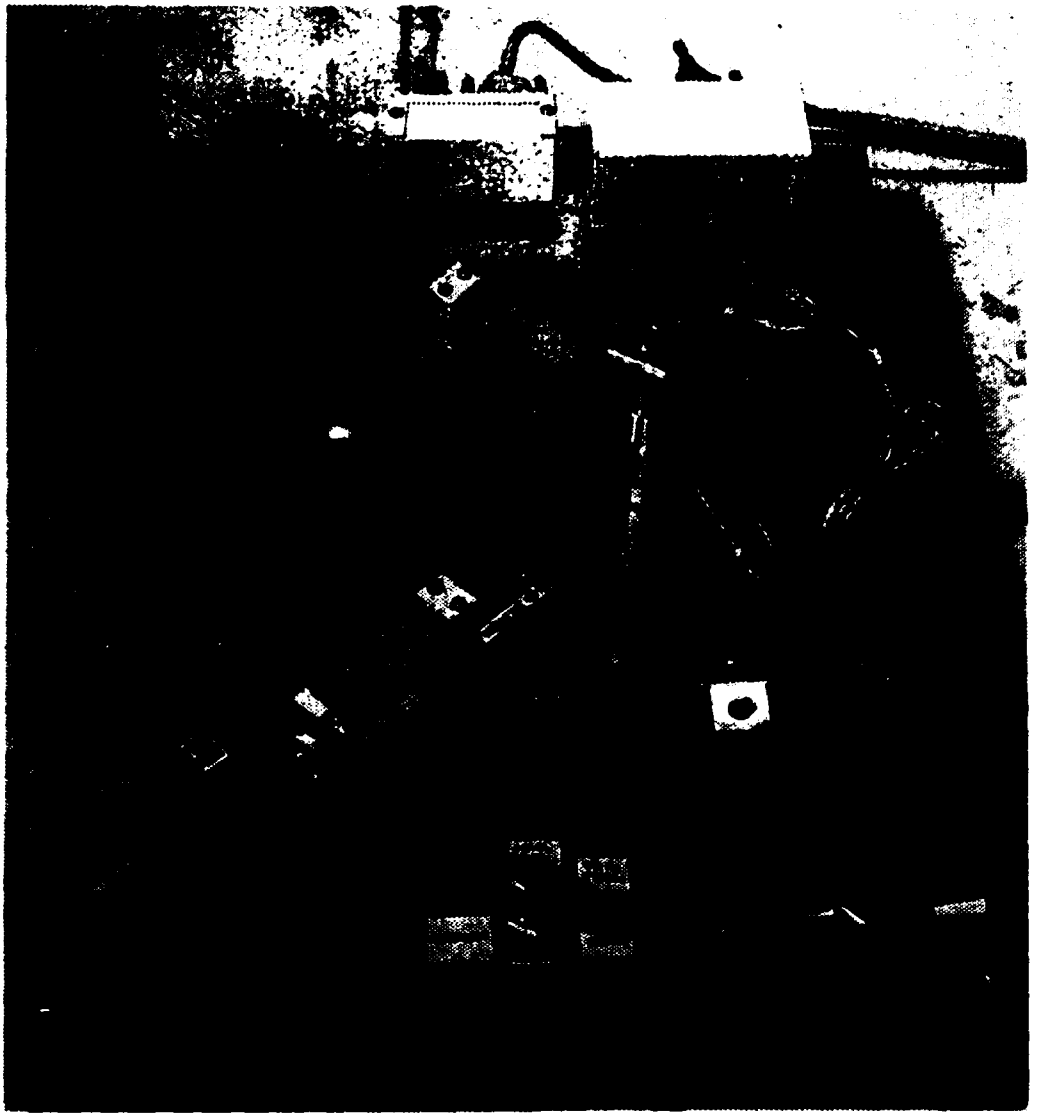
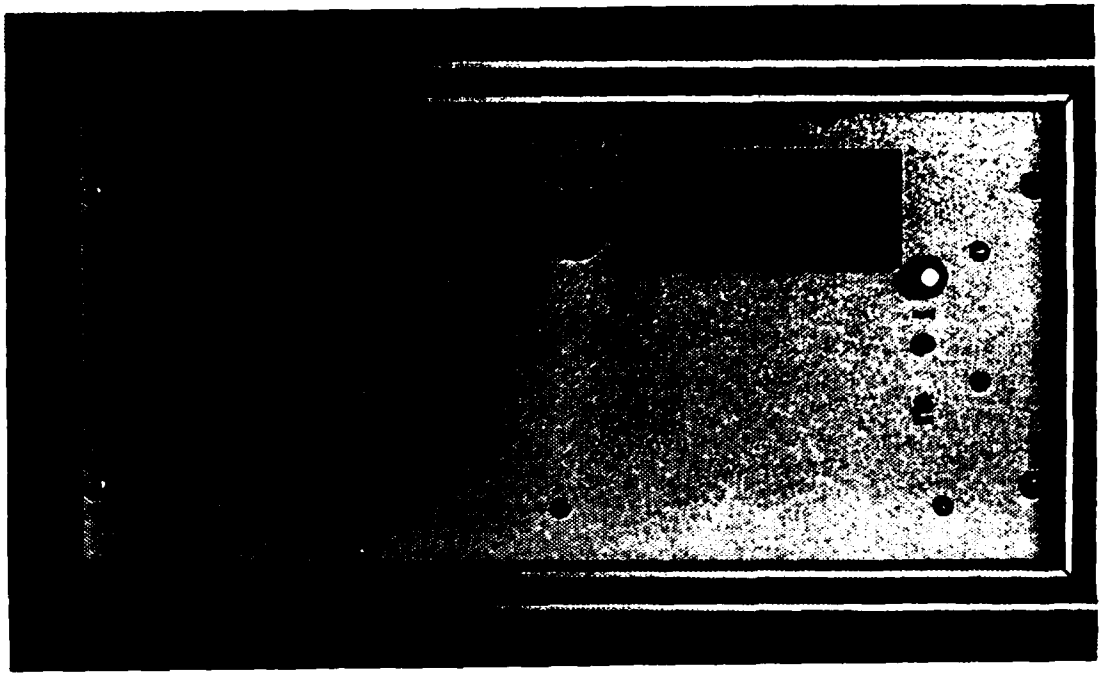


Figure 6 - 12.3

Photograph of the Front Panel of the Electronics Box

To the upper left is seen the switch that chooses either heating or cooling. To its right is the switch that chooses between displaying the temperature from the analog circuit, the signal from the O_2 probe, the signal from the CO_2 probe or the signal sent to the D/A interface that drives the heater on the digital voltmeter to its right. The next row has, from left to right, the main power on/off light, the heater power on/off light, the pot to manually set the heater power, the switch to choose between thermistor temperature output or thermistor resistance signal output, and the pot that controls the stirrer speed. The third row has, again from the left, the main power switch, the heater/cooler power switch, the manual or computer control selector switch and to the extreme right the stirrer on/off switch.



measuring circuit. If you do, an erroneous signal of such magnitude will be sent to the computer that temperature control will be lost and the experiment will be ruined. During computer control, the temperature is plotted on the computer screen as a function of time, and the temperature may also be directly read digitally from the computer screen.

The software that we have supplied uses the resistance measuring circuit and the resistance temperature correlation equation for the particular thermistor that is being used. The resistance circuit may be selected by throwing a switch to its indicated position (R). The resistance measuring circuit generates a voltage which when multiplied by 25000 gives the thermistor resistance directly. This multiplication is done by the software. The software then uses this resistance to calculate the temperature of the specific thermistor that is being used from its correlation coefficients, which can be entered into the software during startup.

The resistance circuit coupled with the computer makes the system more flexible and usable above and below the 25 to 50°C range of the direct temperature measuring circuit even as it makes the measurement more accurate. However, below 15°C (or above 50°C) the output of the resistance measuring circuit will exceed the maximum permissible input to the A/D converter. If it is necessary to work below 15°C (or above 50°C), please contact Princeton Scientific Enterprises, Inc. to have the circuit modified.

The stirrer is driven by a small dc motor that is driven in a novel manner which we have found improves mixing. It rotates in one direction for ninety cycles of the sixty cycle ac line voltage source, then stops for ten cycles. It then operates for ninety cycles in the reverse direction, stops for ten cycles, reverses and repeats the sequence until manually shut off by a switch on the electronics box.

A digital voltmeter on the electronics box may be switched to read voltages from four points on the equipment. It has a range of ±2 v. In position 1, it reads the voltage being sent to an A/D converter by whichever temperature circuit is in use. If the temperature circuit is in use, multiplying the reading by 100 will give the temperature directly. If the resistance circuit is in use, as it must be when under computer control - see above, multiplying by 25000 will give the thermistor resistance. The thermistor has a negative resistance coefficient, thus an increase in temperature causes a decrease in resistance.

Position 2 of the meter switch reads the voltage signal that the O_2 electrochemical probe is sending to its A/D converter and position 3 reads the voltage that the CO_2 probe is sending to its converter. In the event of a malfunction or during calibration, these voltages can be useful in diagnosing the cause of the difficulty.

Position 4 of the meter switch reads the signal (divided by 10) that is being returned to the electronics box by the computer *via* the D/A converter. This is the signal (analog) that controls the power output of the thermoelectric heater/cooler. The analog voltage signal to the heater control circuit varies over the range -5 v (full on) to +5 v (full off).

The electronics box also contains a small fan to cool the electronics, especially the transistor that drives the heater/cooler, as that transistor dissipates several watts when the equipment is operating.

The dual preamplifier is mounted on the mainframe. It accepts the signals from the O_2 and CO_2 probes (with appropriate input resistances), amplifies them and passes them to the electronics box thence to the computer *via* two of the A/D converters. **The CO_2 probe generates a voltage which is a function of the CO_2 concentration. The internal resistance of the probe is very high, and it requires an even higher input impedance to its preamplifier. We have achieved this by using a FET input I. C. in the non-inverting mode. Gain is set at 4X. There is no terminating resistor on the input to permit the achievement of the highest possible input impedance. This means that the input is vulnerable to damage from static charges when a probe is not plugged into it. THE SHORTING PLUG THAT IS SUPPLIED WITH THIS EQUIPMENT SHOULD BE KEPT PLUGGED INTO ONE OF THE INPUT JACKS AT ALL TIMES EXCEPT WHEN THE EQUIPMENT IS IN USE WITH THE CO_2 PROBE PLUGGED INTO THE PREAMPLIFIER. LEAVE THE SHORTING PLUG IN WHILE PLUGGING IN THE PROBE, THEN REMOVE THE SHORTING PLUG. WHEN REMOVING THE PROBE FROM THE PREAMPLIFIER, PLUG THE SHORTING PLUG IN FIRST, THEN AND ONLY THEN UNPLUG THE CO_2 PROBE.**

The O_2 probe does not generate a voltage, but rather it exhibits a conductance that changes with the O_2 concentration. It requires a polarizing voltage of -0.8 v, which is supplied by the electronics. It's preamp is configured as a current to voltage converter. It has a 200 MΩ input impedance and as a result requires no shorting plug. The output signal from this preamp is equal to the input current multiplied by 200 MΩ. With its input port open, its output should be zero within one or two mv. If the input is shorted, the amplifier will saturate.

Section 6 - 13: The Software.

Before running the program, assemble the system. The syringes should be filled with the appropriate solutions and inserted into the reactor. The electrochemical probes should be prepared (fitted with new tips and filled with electrolytes if needed) and inserted into the reactor. The top of the chamber should be assembled with the thermistor in place and attached to the thermostatted reactor holder. The power supply switch and the main power switch on the electronics box should be switched on before starting. The gas supplies should be opened, their pressures equalized at approximately 1 psig, connections checked for leaks and the flowmeter set at approximately 30.

Beginning the Program

To start the program, turn the computer on and bring up the c:\> prompt. Then type "Nbld5" and press "enter". The green main screen will come up with PSE Blood Oxygen Analyzer at the top and five readings (all zero) at the bottom. These readings are temperature (in degrees Celsius), blood CO_2 and O_2 pressures (in Torrs), and total quantities of H_2O_2 and NaOH injected (in microliters). The screen also has two boxes for the program to ask questions and display information. You will be asked five questions before you are ready to begin.

1. The computer will first ask you for the temperature at which you would like to keep the system. Type in your target temperature and press return (*e.g.* 37 <RET>).

2. Then the system will ask you for the Barometric pressure in Torrs. Type in the proper value and press return (*e.g.* 760 <RET>). (Note: If you are using a dry gas supply and humidifying the gas on its way to the reactor, reduce the barometric pressure by the vapor pressure of water at the humidifying temperature and input this value for barometric pressure. If the reactor is operated at the temperature of humidification, this value will give correct results. Barometric pressure is used only in calibrating the electrochemical probes. That calibration uses barometric pressure to change fraction of gas specie being calibrated in the gas mixture to partial pressure of the mixture. Reducing the input value of barometric pressure by the vapor pressure of water that has been added to the system at the calibration temperature makes the necessary correction. This assumes that the calibration temperature is not less than the humidification temperature. If it is, and if the gas was brought to 100% relative humidity, then use the vapor pressure at the lower reactor temperature. If the reactor temperature is greater

than the humidification temperature, use the vapor pressure of water at the humidification temperature to correct the barometric pressure.)

3. You will then be allowed to change the steps per micro-liter for your syringe. If you wish to use the default value, type "n". If you wish to change this value, type "y". You will then be asked for your value. Type in the proper value and then press return (*e.g.* 754.2 <RET>). If you do not type in a value, the computer will use the default value.

4. You will then be asked if you wish to change any of the coefficients for the PID control algorithm (*i. e.* system gain, sampling time, integral time, and differential time). If you wish to keep them unchanged, type "n". If you wish to change any of them, type "y". If you type "y", you will then be asked individually if you wish to change each one. If you do not wish to change the one currently shown, type "n". If you wish to change the one currently shown, type "y". You will then be asked to input your new value. Just type in your new value and press return (*e.g.* 10 <RET>). If you do not type in a value, the computer will revert back to the default value.

5. You will be asked if you have a file for your thermistor coefficients or if you have to create a new one. If you previously saved a file, press "1". A new screen will come up with all the data files listed (this will also include all files which hold data from previous experiments as well as coefficient data). Type in the name of the proper file and press return (*e.g.* Bcoeff <RET>). You do not have to type in the extension name (*i.e.* ".dat"). The computer automatically adds ".dat" to the end of the file's name. The main screen will then come on again. The computer will list the just loaded coefficient values on the screen, and it will ask you if you wish to change these values. If you wish to keep these values as they appear, press "n". If you wish to change these values, press "y". You will then be asked if you wish to change each coefficient individually. To keep the coefficient as is, just type "n". To change the value of the coefficient, type "y". You will then be asked to input the new value of the coefficient. Just type in the new value (you can use scientific notation) and press return (i.e.: 1.38e-7 <RET>). If you do not input a value, the computer will keep its value unchanged. After checking each coefficient, the computer will save the values of the coefficients in the same file they came from. If you ues scientific notation, use "E" for the exponent, not the "D" for double precision.

Note: Be careful not to load a data file which contains data from a previous temperature or experiment run. The

coefficient data is loaded from and saved into the same file, so you would lose all your previous data.

After you have answered all five questions, you are ready to begin temperature monitoring. At this time, the computer will remind you to turn the heater on and place it in computer control. These switches should be checked on the electronics box to be sure they are in the proper position. Also, the stirrer should be turned on. Once everything is turned on and all switches are at their proper setting, you are ready to begin. You begin by striking any key (except any F# key). There will be a ten second delay while the computer takes its first two readings for the temperature monitoring. (The computer tells you it is adjusting temperature.) The main menu will then appear. In the top box, you will be reminded of the two special function keys (F2 and F3), and in the user action box, you will be given the main experiment menu. Simply strike the key of whatever exercise you wish to perform (*e.g.* strike "1" to run the temperature experiment, strike "2" to run the blood experiment and so on). All these routines as well as the two special function keys will be discussed individually.

Special Function Keys

There are six special function keys, two which are active during the entire temperature monitoring (F2 and F3)and four which are active only during the actual blood analyzing experiment (F5, F6, F7 and F8). Each one will be discussed individually.

The F2 key is used to save data to a file. It is useful after having run temperature monitoring or blood oxygenation experiments. After pressing F2 the main computer screen is displayed and you are told the experiment is now finished. The computer asks you if you wish to save your data to a file. If you strike "n", the main menu is brought up. If you strike "y", the computer asks you for the name of your data file. Type in any name you wish (no spaces) up to a maximum of eight letters (the computer will accept no more than eight) and a minimum of one letter (the computer will not accept a blank name). **Do not** add an extension name. The program automatically adds ".dat" to the end of your data file. After typing in the file's name, strike return and the data will be saved. If you decide not to save the data, just strike F2 again and this time answer "n" when asked if you wish to save the file. When you are finished, you return to the main menu.

Note: Data for saving to a file is only generated during a temperature monitoring run or a blood oxygenation experiment. If you strike F2 during any time other than during one of those experiments, the data from the last experiment run will be saved.

The F3 key is used to change the system coefficients (the proportional gain, sampling time, integral time, and differential time) and/or the thermistor coefficients. By pressing F3, the main screen comes up and you are brought to question #4 of "Beginning the program". Just answer questions #4 and #5 normally and then the main menu will appear again.

The F5 and F6 keys are used during the blood analyzing experiment to increase or decrease the amount of NaOH and H_2O_2 injected during each step of the experiment. The amount per injection is multiplied by the square root of 2 every time you strike F5, and the amount per injection is divided by the square root of 2 every time you strike F6. The NaOH and H_2O_2 are increased or decreased together with these keys.

The F7 and F8 keys are used to increase the quantity of NaOH injected per injection. F7 increases the NaOH per injection by a factor of the square root of 2, and F8 decreases it by the same factor. Using these keys in conjunction with F5 and F6 permits one to increase either injection quantity individually.

Routines

Temperature Monitoring (Graphing)

The purpose of this routine is to graph the temperature monitoring of the system. To select it, strike "1" at the main menu. When you strike "1", the graphing screen will appear. The bottom axis is the time. Each line corresponds to 600 seconds (10 minutes) and the 0, 3000, 6000, and 9000 seconds marks are labeled. On the left ordinate axis, the temperature is marked from 25 to 45 degrees Celsius with each mark labeled and equal to two degrees Celsius. The right ordinate axis is for use during the blood analyzing experiment. It measures gas pressure in Torrs, and it is marked for 0 to 250 Torr with each tic mark labeled in increments of 25 Torr. At the top of the screen is a reminder of the function of the F2 and F3 function keys, the time elapsed for the experiment (in seconds), the signal output to the heater (in volts), the temperature (in degrees Celsius), and the set temperature (in degrees Celsius). The computer graphs two things during the temperature monitoring routine. First is the temperature itself. This will be graphed in black. Appearing

around the 27 degree mark will also be a green line. This is the graph of the signal output to the heater. This signal ranges from -5 Volts to +5 Volts. These values correspond to the 26 and 28 degrees Celsius marks on the temperature scale with 27 degrees Celsius representing 0 Volts.

The experiment will run a maximum of 7200 seconds (two hours), at which time the computer will tell you that it is complete. You may end the experiment early by striking the F2 or F3 keys. Upon striking either key, the main screen will appear and you will able to perform the task of the function key you selected. After the time has run out, the computer will tell you to strike the F2 key. **Be sure that if you wish to save the data, you strike the F2 key. If you strike the F3 key and then start another experiment before striking F2, all data will be lost.** After the computer performs the function of whichever key you strike (F2 or F3), the main menu will appear.

Blood Content

Select the blood content routine by striking "2" when you are at the main menu. The purpose of this routine is to measure the blood CO_2 and/or O_2 fraction and print it at the bottom of the main screen.

After pressing "2", you will be asked which one you wish to check. Strike "1" to check the CO_2 fraction, strike "2" to check the O_2 fraction, or strike "3" to exit back to main menu. If you strike "1" or "2", the appropriate blood gas fraction will be monitored and recorded at the bottom of the screen continuously until you strike a key. Striking any key (other than F2 or F3) will bring you back to the blood content menu. When finished monitoring both gas fractions, strike "3" to return to the main menu.

Dispense Liquid

Select dispense liquid by striking "3" when you are at the main menu. The purpose of this routine is to inject NaOH and/or H_2O_2 into the reaction chamber. After striking "3", you will be asked which liquid you wish to inject. Strike "1" to inject NaOH, strike "2" to inject H_2O_2, or strike "3" to exit back to the main menu. After selecting the proper liquid, you will be asked how many microliters you wish to inject. Enter the amount and press return (*e.g.* 12.5 <RET>). The system will inject the amount of liquid you entered, and then it will ask you if you wish to inject more of the same liquid. Strike "y" to inject the

same liquid or "n" to go back to the dispense liquid menu. After you are finished injecting liquid, press "3" at the dispense liquid menu to return to the main menu.

Exit

Select exit by striking "4" when you are at the main menu. The purpose of this routine is to leave the program. After striking "4", the computer will go through the rewind pumps routine to make sure the pumps are in proper storage position. Just follow the instructions for the Rewind Pumps routine below and strike "3" to exit when you are satisfied with the pumps storage position. When you strike "3" you will exit the program.

Calibrate

Select calibrate by striking "5" when you are at the main menu. The purpose of this routine is to calibrate the CO_2 and O_2 probes. When you strike "5", you are given a choice of (1) Calibrate Slope and Intercept, (2) Input the Slope and Intercept, or (3) Check the current slope and intercept values. Select the routine you wish to perform by striking the appropriate key.

To select calibrate slope and intercept, strike "1". The purpose of this routine is to calibrate the probes by running gases of different percentages of O_2 and N_2 into the chamber and taking measurements on the probes. After striking "1", the computer will ask you which probe you are calibrating. Select "3" if you have taken enough data and you wish the computer to compute the slopes and intercepts. If you still need more data, select the appropriate gas by striking "1" for Oxygen or "2" for Carbon Dioxide.

When you select the appropriate gas, you will be asked for the percentage of the gas. Type in the percentage and hit return (*e.g.* for 21% Oxygen, select oxygen by striking "1" then type 21 <RET> for the percentage). After entering the proper percentage, the calibration graph will appear. A graph will appear with axes marked but not labeled. At the top of the page is the present temperature, the target temperature, and the value of the signal from the probe. The purpose of this graph is to watch the signal from the probe until it levels out. Make sure the appropriate gas is in the chamber and then wait for the probe signal to level out. (Readings are taken every 30 s, thus exercise patience until you are certain that a steady signal has been obtained.) When the signal levels out, strike any key (except any F# key) to

accept the present signal value as the data point for the entered gas percentage. When you strike a key, you will go back to the main screen , and you will be given the above choice again to calibrate (1) Oxygen, (2) Carbon Dioxide or (3) End Calibration.

If you still want to take more data points choose either "1" or "2" for the appropriate gas. (For the O_2 probe, the system performs a linear regression of the signal versus the pressure of O_2 in the reactor. For the CO_2 probe, the system performs a linear regression of the signal versus the natural logarithm of the CO_2 pressure in the reactor. The pressure of the calibration gas is calculated by multiplying the fraction of that gas in the gas mixture by the barometric pressure. If the gas comes from a high pressure cylinder, it is dry at atmospheric pressure. If it is not humidified prior to use in the calibration, the product of gas fraction times barometric pressure gives the correct pressure. If however the gas is humidified, reduce the barometric pressure that is input to the program by the partial pressure of the water added, and enter this as the barometric pressure when you start the program. The pressure calculation will then be correct. You must take at least two calibration points at different fractions of each calibration gas for the program to function. This is because the regressions must have at least two points each.) If you have enough data, strike "3" to select end calibration. If you do not have enough data points for one of the gases, the computer will remind you by displaying a message which reads "You must take at least two data points for O_2 (or CO_2)". The message will appear for two seconds. Then the above selection of oxygen, carbon dioxide and end calibration will appear. Continue the above process until you have enough data. When you have the required amount of data and then strike "3", the computer will compute and then display the slope and intercept of the linear regression of the O_2 signal versus the O_2 pressure. It is a good idea to record this value for future use. Then, after you strike any key (other than any F# key) the computer will compute and then display the slope and intercept of the regression of the CO_2 signal versus the natural logarithm of the CO_2 pressure. Again, it is a good idea to record this value.

After you have recorded the carbon dioxide information, strike any key (except any F# key) to exit calibration and return to the main menu. Unless you make a subsequent change, the computer will use these values in the experiment until the computer program is turned off.

Select the option to input the regression coefficients by striking "2" at the first calibration menu. The purpose of this routine is to allow you to enter a new value for either the CO_2 or O_2 slope and intercept. After striking "2", the computer will

ask you if you wish to enter the O_2 values. To input the O_2 values, strike "y". To leave them as they are strike "n". If you strike "y", you will be first asked to input the slope of your O_2 curve and then the intercept of your O_2 curve. Enter each value and hit return (*e.g.* if the slope is 3 and the intercept 6, type 3<RET> and 6<RET>). After entering the O_2 slope and intercept, or if you selected "n" above, you will be asked if you wish to enter the CO_2 values. Follow the same procedure as with the O_2 values. After you are finished with the CO_2 values, the computer will automatically return to the main menu.

To select check the current slope and intercept value, strike "3" at the first calibration menu. The computer will then display the current value of the CO_2 and O_2 slopes and intercepts. After checking the value, strike any key (except any F# key) to return to the main menu.

Experiment

To select the experiment, strike "6" at the main menu. The purpose of this routine is to run a blood oxygenation experiment. After you strike "6", the computer will tell you to be sure the temperature is stable before you begin the experiment. (This program has been modified to run in two stages, prestage and experiment. Prestage can be used to attain temperature equilibrium if that is desired or necessary.) Monitor the temperature in the lower left hand corner of the screen. Once it is stable near the target temperature, strike any key (except any F# key) to continue. (A better way to get the temperature at the appropriate value is to select "1" at the main menu and run the temperature graphing routine until the temperature is stable at the target temperature.) The computer will then ask how much H_2O_2 you would like to inject per injection. Type in the proper amount (in microliters) and hit return. Then you will be asked the amount of NaOH per injection. Type in the proper amount (in microliters) and hit return. Then you will be asked how long to run the experiment. Type in the proper time (in seconds) and hit return. The maximum time is 7200 seconds. If you enter a time longer than 7200 seconds, the computer will automatically make it 7200 seconds. Then, in the top box, you will be reminded you can prematurely end the experiment by striking the F2 or F3 key. Strike any key (except any F# key) to begin the experiment. This will start the experimental prestage, and the experiment screen - graph plus messages - will appear. The program will withdraw the plungers on both syringe pumps 0.15 µl. This is to prevent reagents bleeding into the reactor when the blood is added but prior to the start of the experiment. The program will now continue to control the temperature as it begins its normal

sequence of monitoring and reporting the O_2 and CO_2 pressures as well as the temperature. These values will be reported and graphed, and they will become part of the data file. During this period the reactor should be purged with the chosen blanket gas until the blood is injected into the chamber. Just before the blood is injected into the chamber, switch the blanket gas to the sweep position. When the system is stable and you are ready, inject the blood slowly into the reactor. Monitor the screen until the system is stable. When you are ready, strike any key (except any F# key) to start the experiment. The experiment will then begin after 30 s with an injection of H_2O_2. The initial injection of either H_2O_2 or NaOH will move the corresponding pump 0.15 µl plus the intended injection quantity. This removes the dead space in each syringe caused by the earlier 0.15 µl pull back.

The experiment graph is the same as the temperature graph with more information. At the top is a reminder of the purpose of all six function keys, F2, F3, F5, F6, F7 and F8. Just below that is the present temperature and target temperature. Just below that is the amount of H_2O_2 and NaOH injected into the chamber per injection. Just below that is the total amount of H_2O_2 and NaOH injected during the current experiment up to the current time. To the right of this information are four more pieces of information. The topmost is the time elapsed from the start of the experiment. Next is the number of the last data point taken. Next is the last O_2 pressure data taken. Finally, under that is the last CO_2 pressure data taken. There are three pieces of data graphed every time a data point is taken, the temperature is graphed in black, CO_2 pressure in white, and the O_2 pressure in blue. Data points are only taken after the CO_2 pressure as been adjusted by injections of NaOH to be equal to or less than its starting value. The experiment will continue until one of five things occur. (1) You press F2. (2) You press F3. (3) The experiment time runs out. (4) The H_2O_2 runs out. (5) The NaOH runs out. If 3,4, or 5 occur, the computer displays a message telling what occurred and asking you to strike F2 to continue. Therefore, the only two ways to exit the experiment graphing screen is by striking the F2 or F3 key. When you strike one of these keys, you perform the function listed earlier, and then you return to the main menu.

Note: Empty the reactor before rewinding the pumps, otherwise reactor fluid will be drawn into the pump syringes.

Rewind Pumps

To select rewind pumps, strike "7" at the main menu. Rewind pumps is also automatically run when you are exciting the program. The purpose of rewind pumps is to bring the syringes back to a position where they are ready to be refilled. The first thing you must select is which pump to rewind. To rewind the NaOH pump, strike "1". To rewind the H_2O_2 pump, strike "2". To exit back to the main menu (or from the program) strike "3". After selecting the pump, the procedure is the same regardless of the pump. First the computer asks if you need to rewind the pump beyond where it started on this run. If you strike "n", the motor will automatically shut off when it reaches the home position. If you strike "y", the motor will not shut off until you tell it to stop. Then you are told to hit any key (except any F# key) to start rewinding. Once it starts rewinding, it will not stop until you strike a key or it reaches its home position and you told it not to rewind beyond its home position. When the motor stops rewinding, the computer will ask you if you wish to continue rewinding the same motor. If you strike "y", the computer will return to the step where it asks if you wish to rewind beyond the home position. If you strike "n", the computer returns to the first rewind pumps menu. Continue the above process until both pumps have been sufficiently rewound. Then type "3" at the first rewind pumps menu to exit rewind pumps.

Print Data

To select print data, strike "8" at the main menu. The purpose of this routine is to print a data file to the screen or to a printer. The first question the computer asks is the type of data file. Is it from a (1) experiment run or (2) a temperature monitoring run. Strike "1" or "2" to select the proper file type. After striking "1" or "2", a new screen comes up with all the data files listed. The computer asks you to type the name of your data file. Type the name of one of the files (do not add ".dat", the computer does it for you) and hit return. The file name must be between one and eight letters (the computer will not accept anything else). If you type a file not there, the computer puts up a file not found message for two seconds and then ask you for the name of your file again. After you type in the appropriate name, the computer will ask you if you wish to print to the screen or to a printer. To print to the screen, strike "1". The computer will then print the data a screen at a time (pausing until you hit any key except any F# key). To print to a printer, strike "2". You disconnect the blood instrument from the printer port and connect a printer to the computer before striking "2". If a printer is not hooked up, the computer will tell you it is unable to find a printer.

Strike "a" to abort printing, or connect the printer and strike any other key (except any F# key) to try again. After printing the file, the computer returns to the main menu. After printing to the printer, be sure to reconnect the printer port to the blood instrument.

Closing Down

After exiting the program, shut off all electrical devices, clean out the syringes, remove the probes, and clean out the chamber.

Section 6 - 14: Some test results:

The system was tested using blood to investigate its performance. Results are shown in figures 6 - 14.1 through 6 - 14.10. (In the data file that is recorded by the software, the first row has the tuning parameters, K_p, T, T_i and T_d. Starting with the next row, the columns are CO_2 signal, v; O_2 signal, v; CO_2 pressure, Torr; O_2 pressure, Torr; Temperature, °C; cumulative volume of H_2O_2 solution injected, µl and cumulative volume of NaOH solution injected, µl.)

Figure 6 - 14.1 is a plot of data recorded during an oxygenation test of blood that was made February 15, 1994. In this test, the target temperature was 37°C, the injected quantities were 1.0µl per injection for both H_2O_2 and NaOH solutions and the solutions were 3% H_2O_2 and 0.5N NaOH. The blood was human blood. It shows the history of the data acquisition. The data are CO_2 pressure, O_2 pressure, temperature, and total volumes of H_2O_2 and NaOH injected, all as functions of time from the start of the test. At the time of this test, the software started data acquisition with the first injection of H_2O_2; data was not recorded during the preconditioning period. Figure 6 - 14.2 shows the temperature variation during that test to a greatly expanded scale. It was held to within ±0.02°C with an overall upward trend of approximately 0.01°C over the 2000 s period of the test. This was well within our target precision of ±0.2°C. Figure 6 - 14.3 shows the variation of CO_2 pressure during the test; also to a greatly expanded scale. It was held to less than ±1 Torr with a slight upward trend (≈ 0.2 Torr) during the test.

Figure 6 - 14.1

Blood Test of February 15, 1994

Whole Blood Equilibrated With

5% CO_2 in N_2 at 22 Deg. C

Oxidizer 3% H_2O_2 Solution; Base 0.5N NaOH

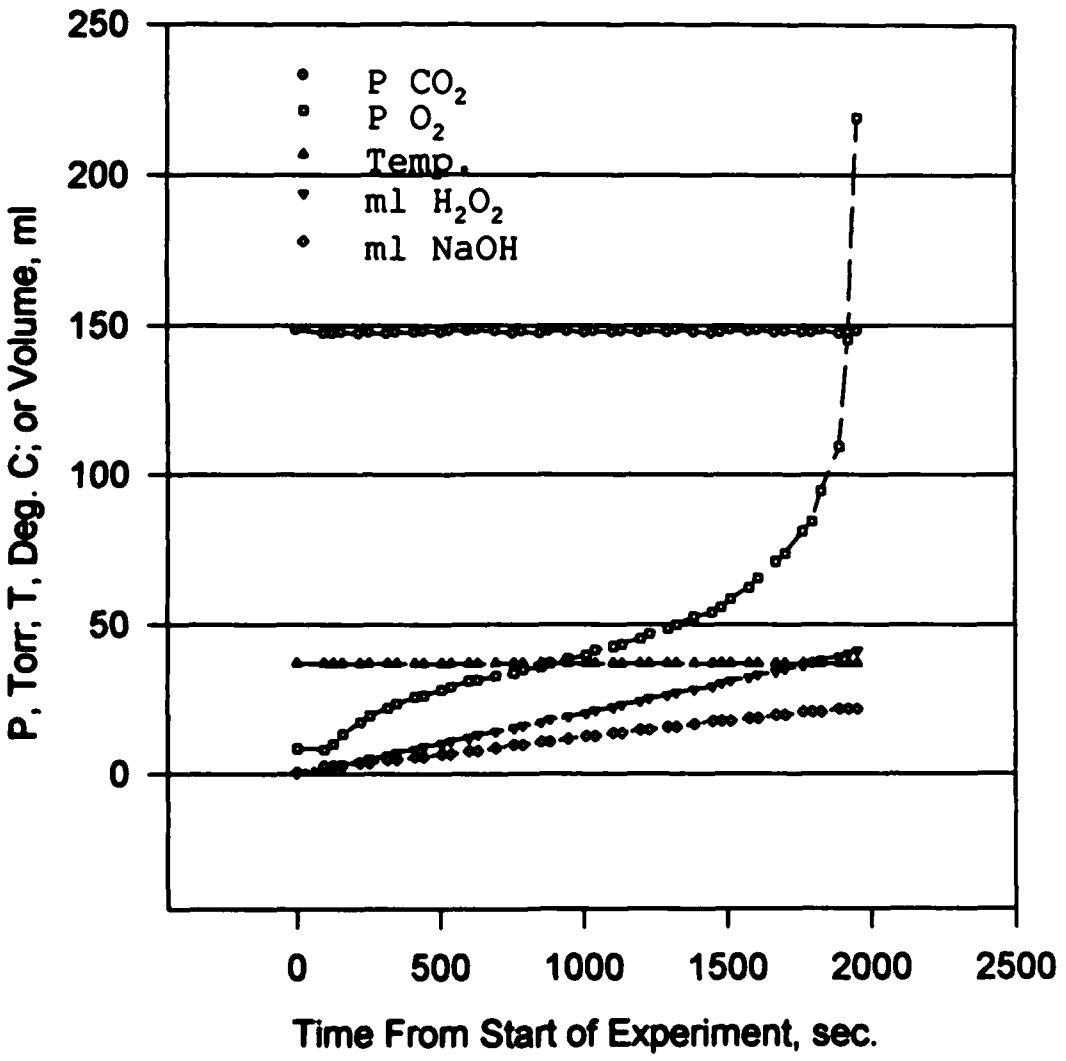


Figure 6 - 14.2

Temperature, Blood Test of February 15, 1994

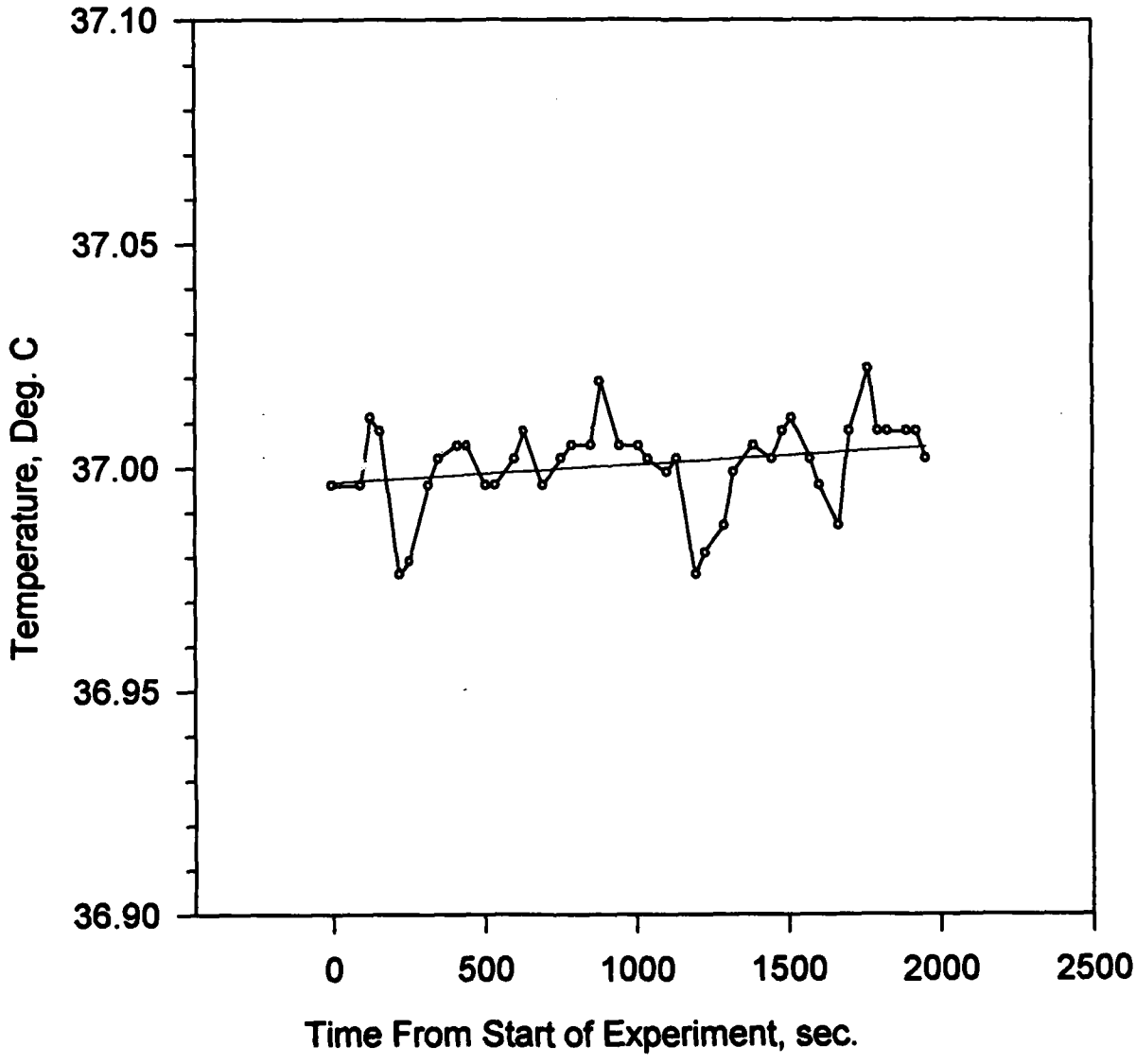


Figure 6 - 14.3

CO_2 Pressure, Blood Test of February 15, 1994

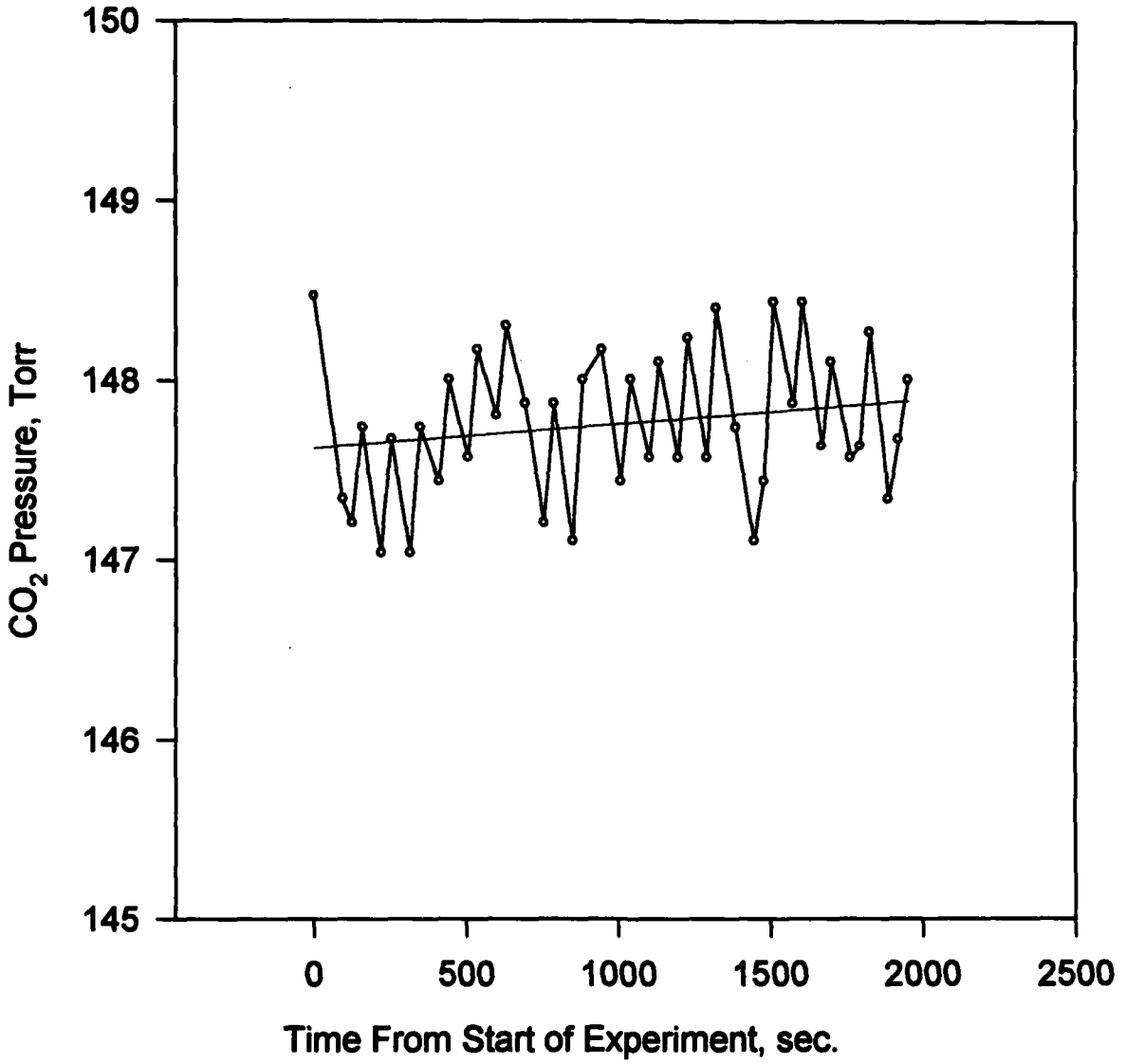


Figure 6 - 14.4 shows the O_2 pressure during the test. It displays an initial rapid increase with the rate lessening with time (injection quantities were constant) until it increases sharply towards the end of the test, as was expected. Figure 6 - 14.5 shows the cumulative volumes of H_2O_2 and NaOH solutions injected during the test. The curve of NaOH injected shows that after some injections of H_2O_2 a single injection of NaOH was required to bring the C_2 pressure back to its original value or less and for others no NaOH injection required. Remember that after an H_2O_2 injection is made no data is recorded until sufficient NaOH injections have been made to return the CO_2 pressure to its original value or less.

Figure 6 - 14.6 shows the oxygenation saturation curve that was generated from these data using the method of Winslow, *et. al.*[7] The data reduction **assumed:**

- Initial hemoglobin saturation, $Y_0 = 0.0$
- Hemoglobin concentration, Hb = 10.0 µmol/ml.
- HbCO concentration = 2%.
- MetHb concentration = 0.5%.
- Initial oxygen content, $O_0 = 0.0$ µmol.
- Electrode factor, F = 1.0.
- Solubility coefficient for O_2 is that of water. α = 0.001018 µmol/Torr µl at 37°C.

The curve, which is reasonable in its general form, shows a maximum fractional saturation of 1.062, which cannot be correct. The error undoubtedly stems from the assumed values of the above parameters that were used in the data reduction.

Figure 6 - 14.7 shows the results of a subsequent test with blood with the modified software that collects data starting with the preconditioning period prior to injection of blood into the reactor. Preconditioning started at time zero. Blood was injected at 331 s. It was at room temperature, and its injection caused the temperature in the reactor to drop to 28.96°C. The drop in temperature is evident on the graph. The blood had been conditioned at room temperature in with gas that was 5% CO_2 in N_2. When the blood was heated in the reactor, this drove the CO_2

Figure 6 - 14.4

O_2 Pressure, Blood Test of February 15, 1994

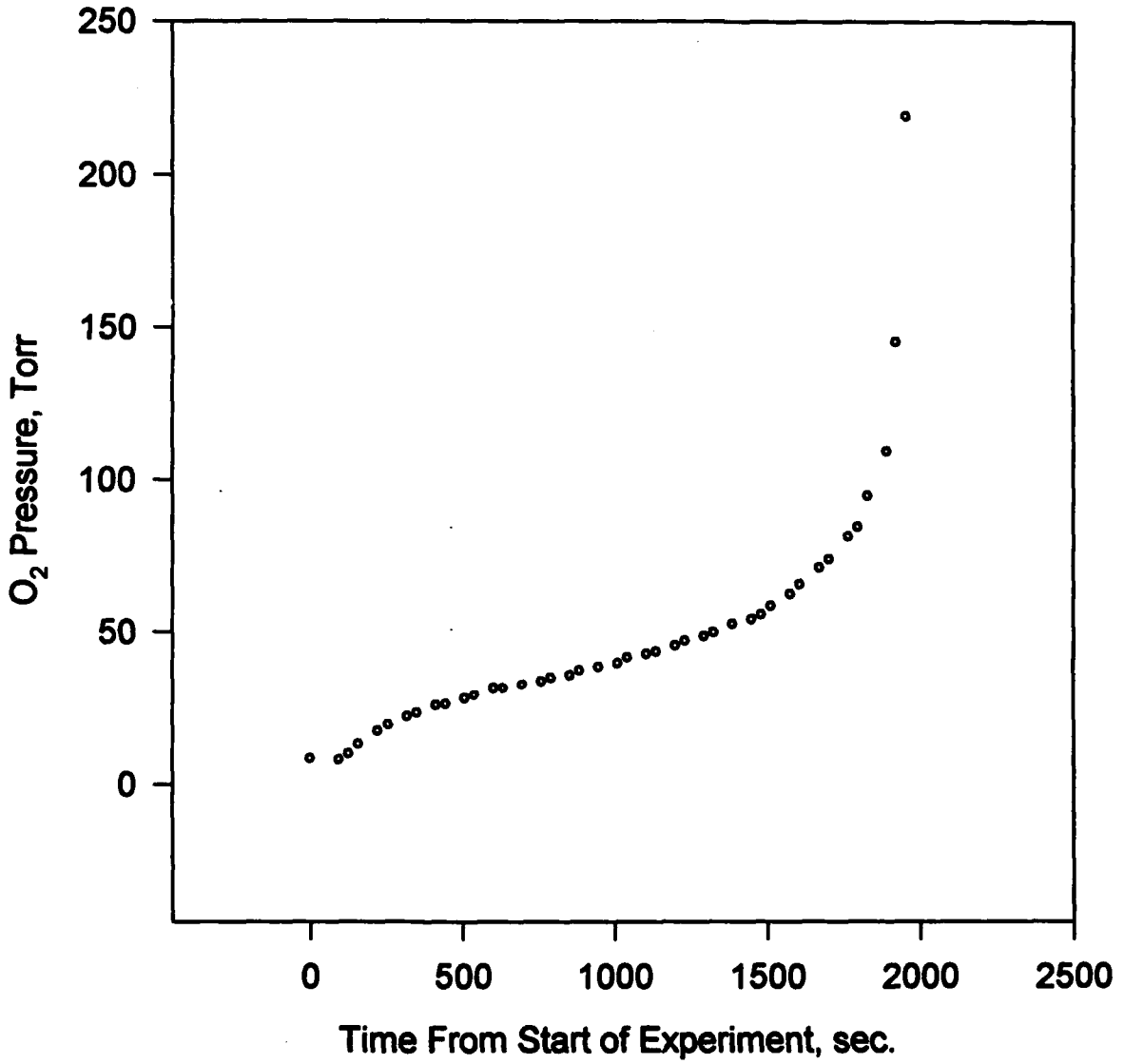


Figure 6 - 14.5

Total Reactant Injected

Blood Test of February 15, 1994

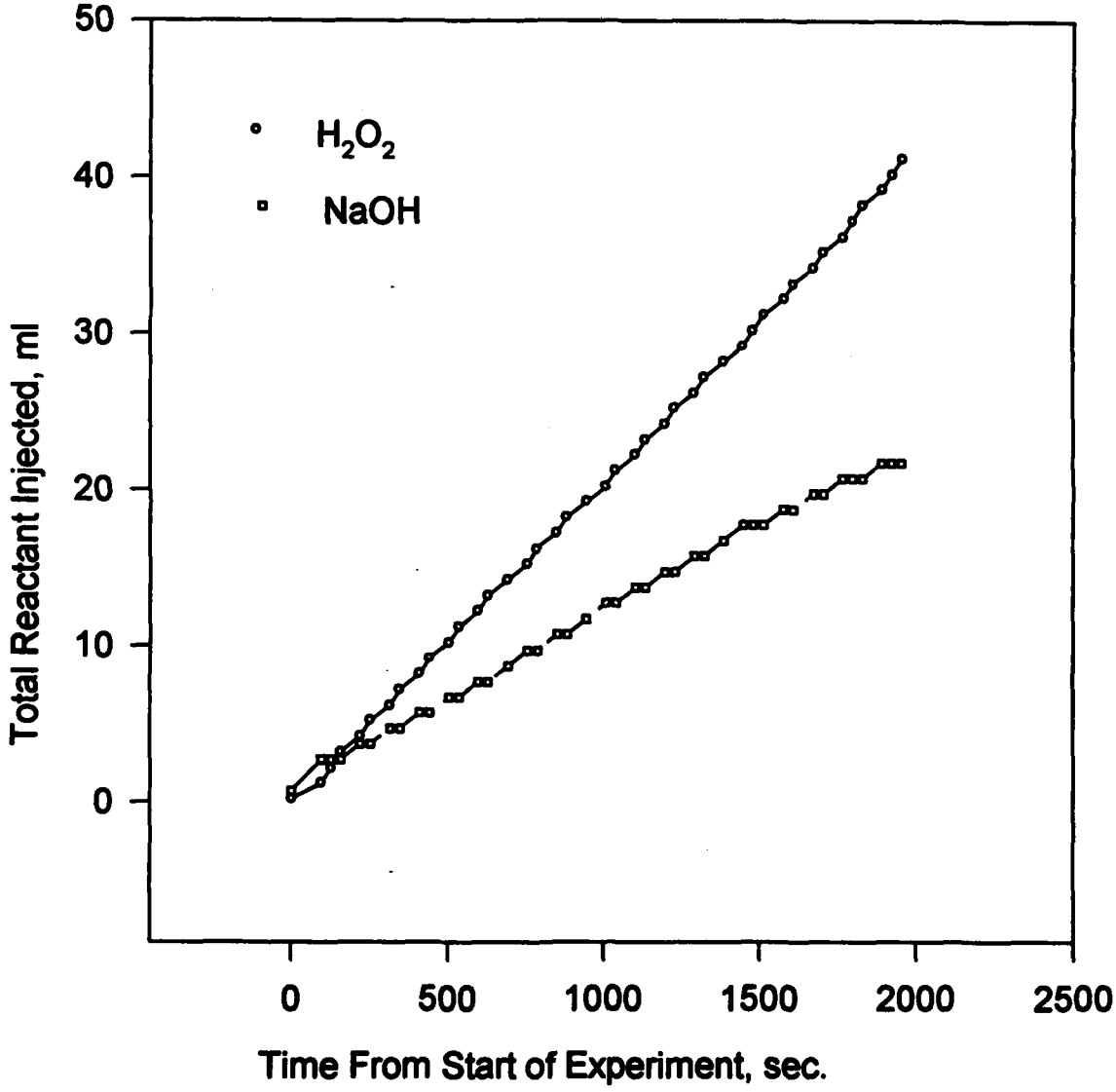


Figure 16 - 14.6

Saturation Curve, Blood Test of February 15, 1994

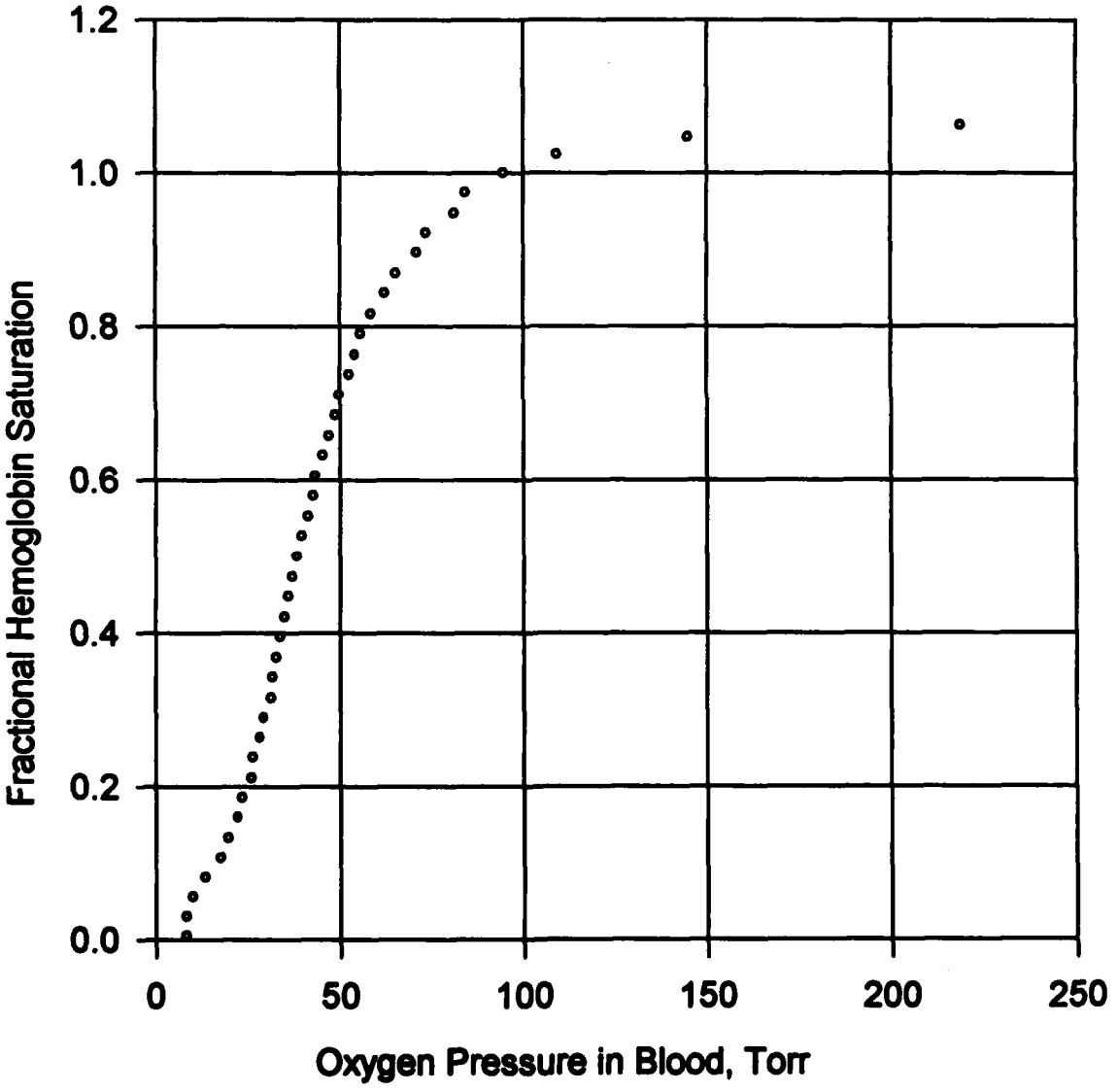
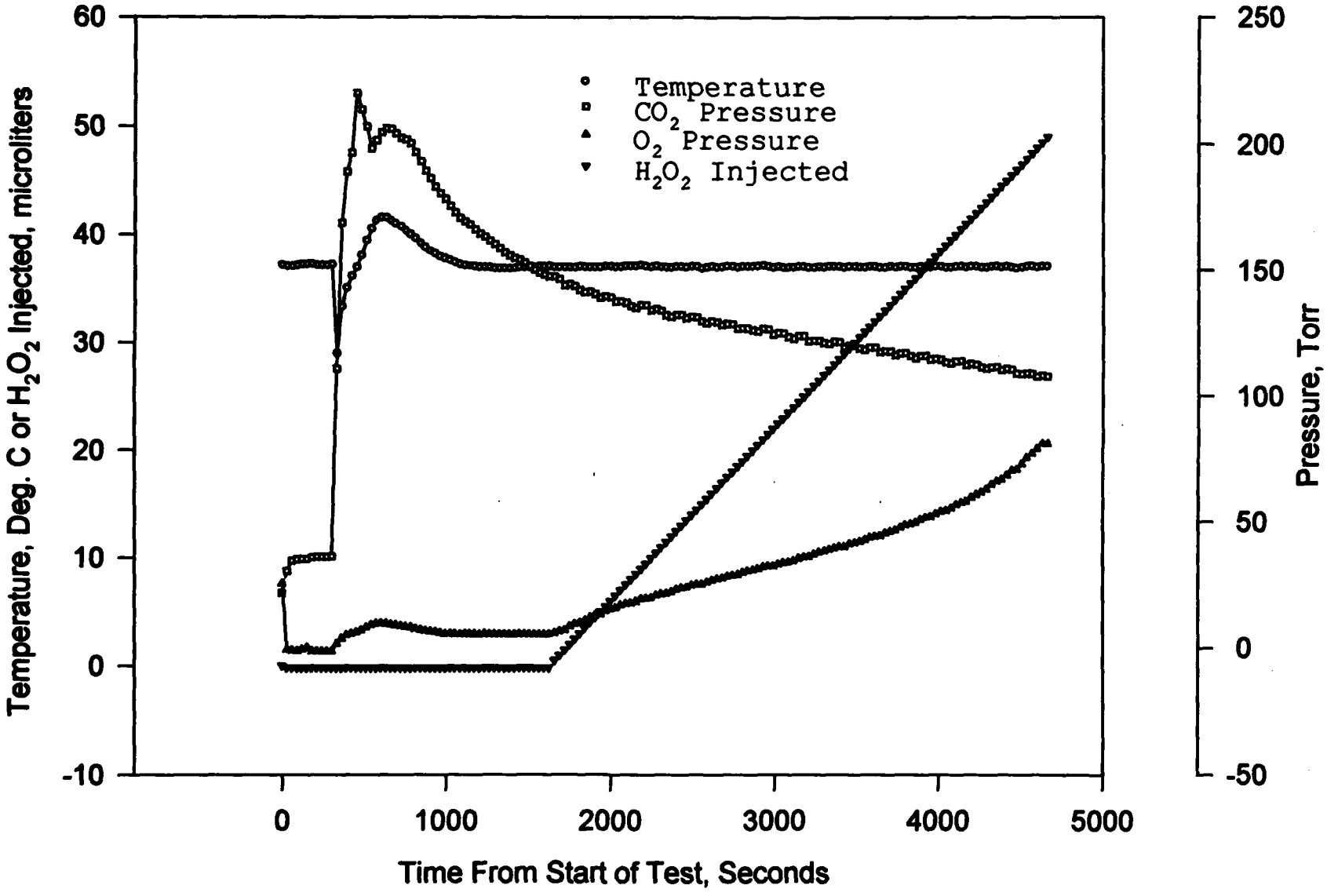


Figure 6 - 14.7

Blood Oxygenation Test of May 25, 1994



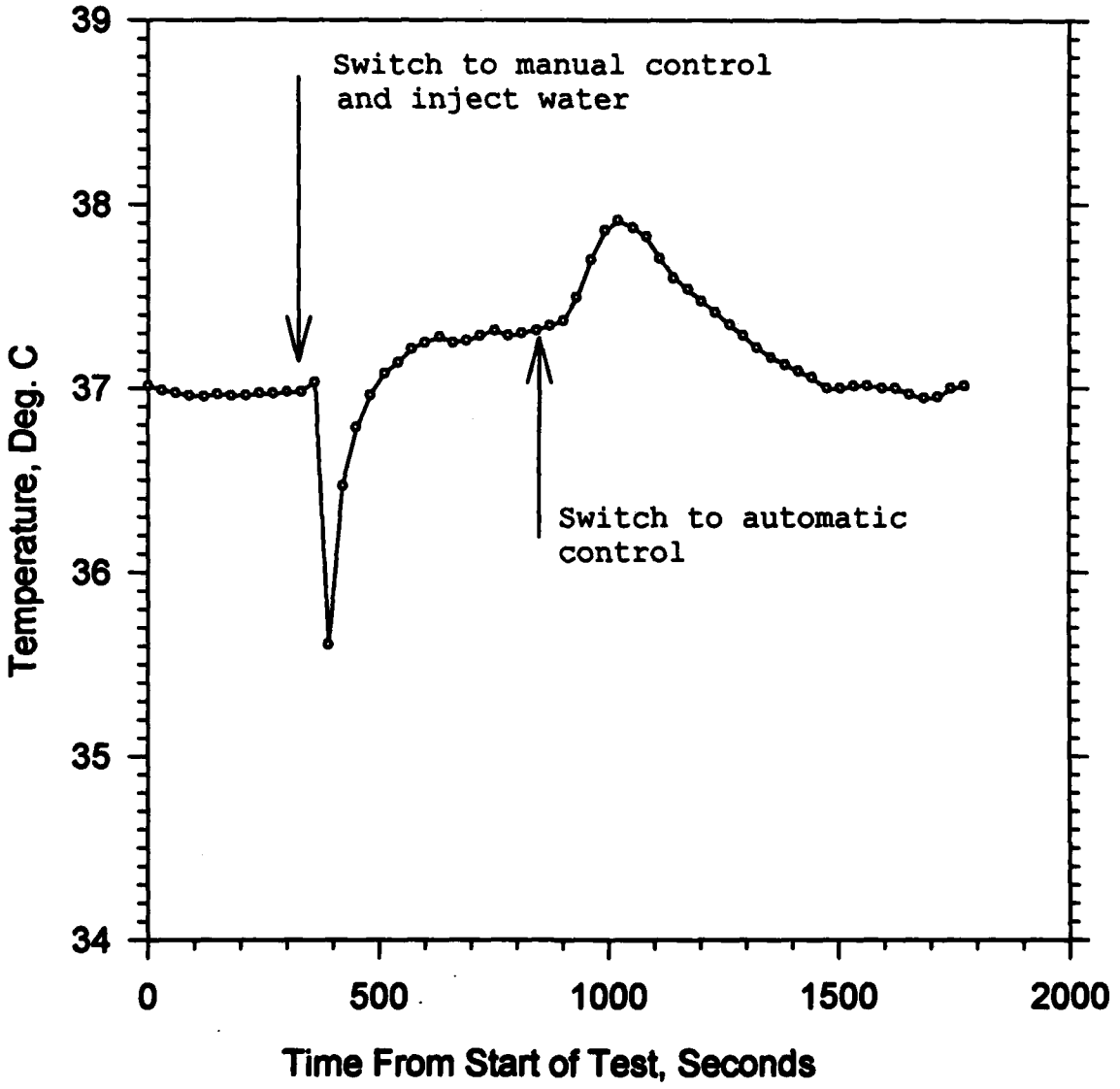
pressure up to 220 Torr. This was far above the pressure in the 5% CO_2 sweep gas, and the pressure started to slowly decrease. The time for it to decrease to equilibrium was too long to wait, and we started injecting H_2O_2 at around 1628 s. Because the CO_2 pressure was continually falling, there were no NaOH injections during this test. The test continued until we exhausted the H_2O_2 solution in the syringe. This occurred just before saturation was reached, as may be seen from the graph. This graph illustrates the result of our modification of the software to record pretest data.

The test also illustrates several other important points. The first is that if the blood is conditioned at a temperature other than that at which the test is to be run, it is important to lower the CO_2 pressure in the conditioning gas to a value below that intended in for the test: the value to be chosen so that the value in the blood at the test temperature is that intended. This lowering of the CO_2 pressure in the conditioning gas can be achieved either by lowering the percentage of CO_2 in the gas or by lowering the total pressure of the gas during conditioning. Far better than either of these methods is to condition the blood at the test temperature with the CO_2 pressure at its intended test value. This helps with the second point. If the blood is not at the test temperature when it is injected into the reactor, it will cause a temperature perturbation that will require significant time to stabilize at the set temperature. The delay is not desirable. If the blood temperature at injection is below the set temperature, it will cause a temperature overshoot during recovery. The magnitude of the overshoot will be proportional to the value of the temperature deficit of the blood at injection, and it may be too large to tolerate for a given test. Thus there is great advantage in conditioning the blood at the test temperature and maintaining it at that temperature until it is loaded into the reactor.

We ran some tests to determine how best to handle temperature mismatches between the set temperature and the injected liquid. Figure 6 - 14.8 shows the results of a test in which we allowed the reactor to stabilize at the set temperature of 37°C, switched to manual control with the power to the heater held at the value that was being called for under automatic control, injected water at a lower temperature and waited for temperature recovery before switching back to automatic control. The water was injected at 361 s. Upon injection of the water, the temperature in the reactor fell to 35.6°C. It gradually recovered under manual control (constant power to the heater) to approximately 37.6°C when the system was switched back to automatic control at about 840 s. The temperature overshot to a

Figure 6 - 14.8

Loading Temperature Test #1, With Water



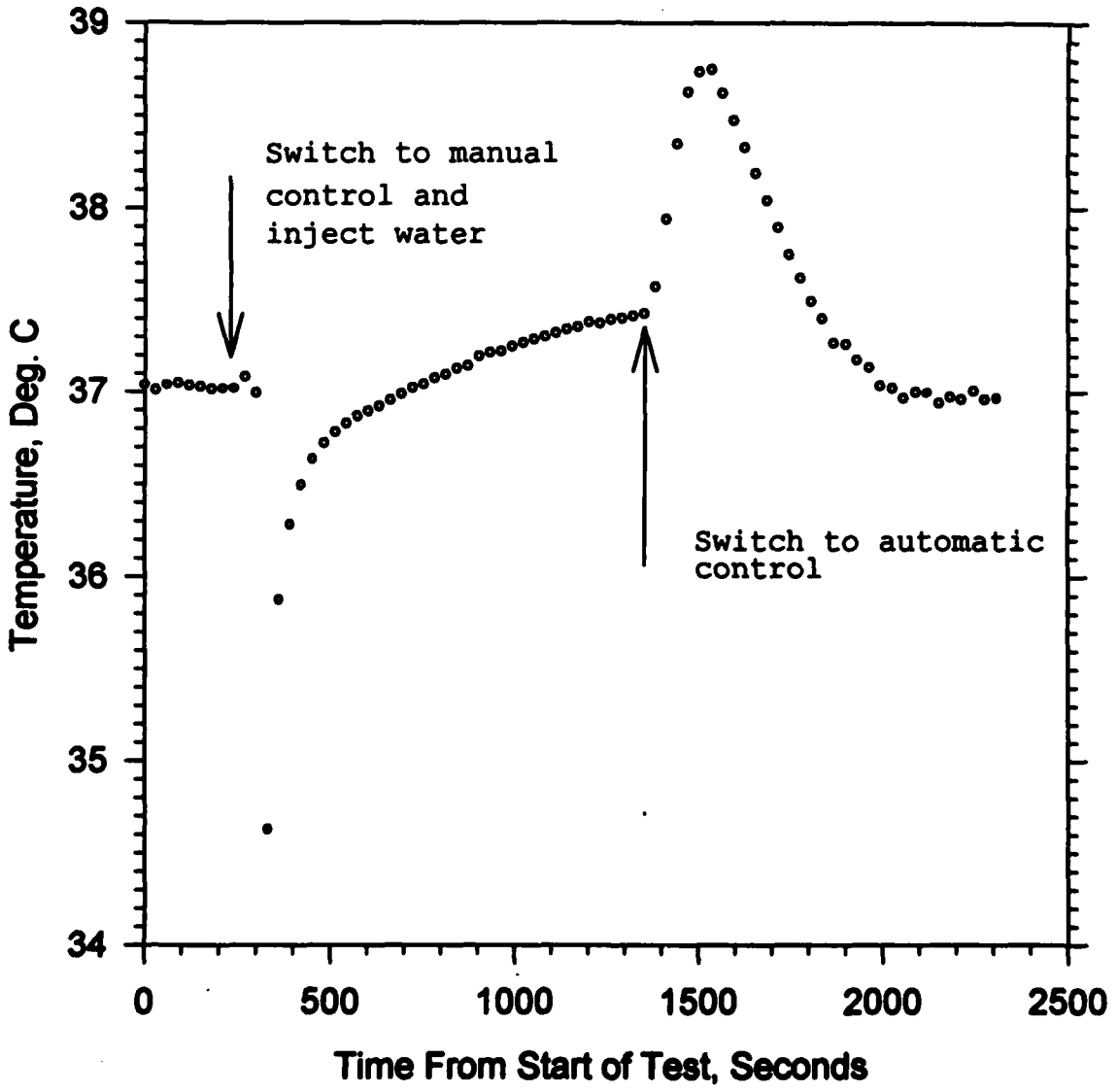
maximum of 37.91°C at 1023 s. It then recovered to within 0.1 °C of the set temperature by 1414 s. Thus, the time from injection until full temperature recovery was 1053 s (17.5 min.). In the case from a restart of the program with the reactor and water starting at 35.5°C, equilibrium at the set temperature was achieved in 654 s (10.9 min.). This suggests that restarting immediately after injection of the blood can speed the attainment of equilibrium at the operating temperature. This will be done at the cost of losing some data during the conditioning period, but this loss can be kept small by switching to the pre test stage after about two min. Alternatively the software can be modified to simulate restart conditions upon injection of the blood. We intend to make this modification, but time did not permit us to make it and test it prior to the deadline for this report.

The best way to reduce the time to temperature equilibrium after injection is to have the blood at the set temperature when it is injected into the reactor. Not when it is removed from the tonometer or other blood conditioner but, rather, when it arrives in the reactor. The smaller the temperature perturbation that is caused by injection, the lower will be the temperature overshoot and the quicker will equilibrium be obtained at the set temperature.

Figure 6 - 14.9 shows the results of another test of temperature recovery after injection of water. In this instance the system was switched to manual control leaving power to the heater at the value at the time of switching. This switch and injection of water were done at 271 s, and the temperature fell to 34.6°C at 331 s. The temperature was allowed to recover to the relatively high value of 37.4°C at 1354 s when the system was returned to automatic control. It was thought that this high temperature might suppress the overshoot caused by return to automatic control, but this was not the case. The temperature shot up to a maximum of 38.7°C at 1535 s, and then it returned to equilibrium at the set temperature at . 93 s, 1722 s after injection. This was probably caused by the data that the control had been collecting during the period of manual control. During that period, the system had been collecting data and generating corrections, but those corrections were not applied to the power input <u>although the control thought that it was.</u> Thus erroneous error corrections were generated, and they became immediately effective upon return to automatic control. A possible fix for this problem is to modify the software to set all errors to zero upon return to automatic control. This will hold the power constant until appropriate errors are generated, and temperature recovery should be much like start up with a temperature initially very close to the set temperature. In any event,

Figure 6 - 14.9

Loading Temperature Test #2, With Water



having the injected fluid temperature as close to the set temperature as possible at the time of injection will be of great advantage.

Figure 6 - 14.10 shows the results of a hurried test that was done with injection and the system at all times under automatic control. It was hurried in that we did not wait for complete return to equilibrium after injection. Injection occurred at 865 s, when the temperature was 37°C. It fell to 35.9°C at 875 s, and it then maximized at 38.5°C at 1136 s. It returned to 37.5°C by 1467 s, 602 s after injection, when the test was terminated. Although we did not wait for full return to 37.0°C, the recovery time promised to be relatively short, reflecting the small temperature drop that occurred upon injection.

These tests show the importance of conditioning the test fluid to the set temperature prior to injection and the recovery times that can be expected if that is not done. They also show the importance of proper temperature during conditioning if one wishes to achieve a specified CO_2 pressure in the test fluid at the set temperature. Finally, they suggest modifications to the software that we will make to improve temperature recovery after injection at non matching temperatures.

Section 7

Conclusions

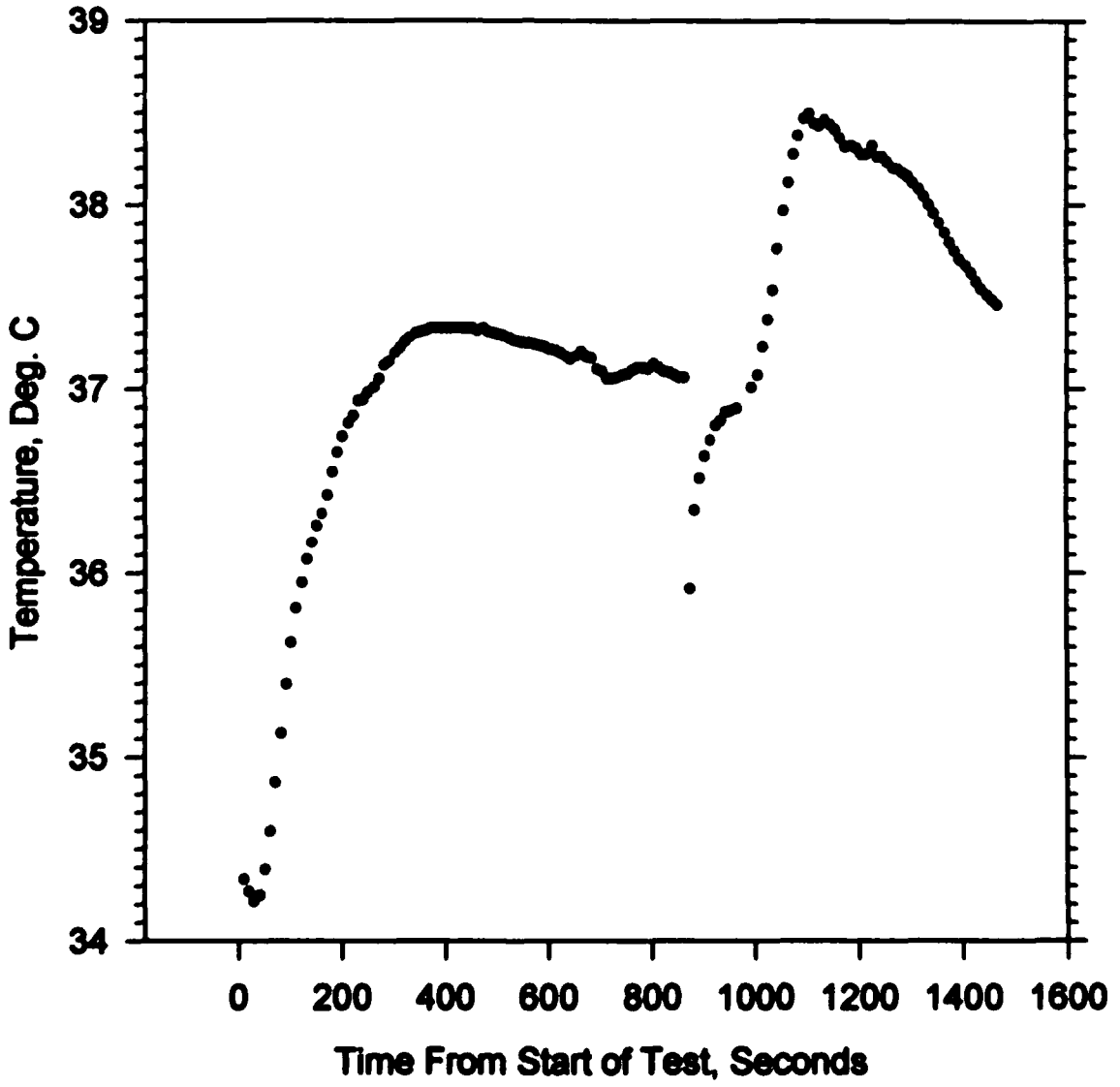
This program has produced a prototype blood oxygenation research instrument that is a significant advance over previous instruments. It uses state of the art technology. It achieves temperature control well within 0.1°C at temperatures either above or below ambient, provides precise digital injection of discrete quantities of reactants, controls CO_2 pressure during testing (and can be readily adapted to provide direct pH control), uses inert quartz reactors, operates under computer control using standard DOS based computers, provides reversing stirring for good mixing at moderate speeds, provides excellent shielding from atmospheric gases and is simple to use. It is a valuable laboratory instrument for its intended purpose.

It requires some modification of its software to improve its operability regarding time that it requires to achieve thermal equilibrium after reloading with a temperature mismatch between

Figure 6 - 14.10

Loading Temperature Test #3, With Water

All Under Automatic Control



injection temperature and set temperature. This will be done and be tested.

It also will benefit from changes to make it into a more commercial configuration. These include compacting it to require less space and provision of enclosures of primarily cosmetic design. This will be done in the future.

The program has produced an instrument that is of immediate utility for blood, hemoglobin and artificial blood research and delivered it to the U. S. Army Medical Research and Development Command, as was the intention of the program.

Section 8

References

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2.Bucek, V. J. 1989. *Control Systems: Continuous and Discrete.* Englewood Cliffs, New Jersey: Prentice Hall.

3.*ibid.,248, eq. 14.2.1.*

4.ibid.,250, eq. 14.2.16.

5.ibid., 250, eqs. 14.2.13, 14.2.14, 14.2.15.

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Appendix A

Thermistor Calibration Data

$$Rt = \exp(A0 + A1/T + A2/T^2 + A3/T^3)$$

A0 = -4.242250582870D+00
A1 = +5.080185453560D+03
A2 = -3.615069577532D+05
A3 = +2.354808245771D+07

TABLE OF ERRORS

CALIBRATION TEMPERATURE DEG. C	CALIBRATION RESISTANCE OHMS	COMPUTED RESISTANCE OHMS	TEMPERATURE ERROR DEG. C
15.00000	22436.00000	22436.11223	-0.00012
25.00000	15041.00000	15040.71477	0.00049
37.00000	9591.60000	9591.89830	-0.00086
50.00000	6091.10000	6090.86728	0.00113
55.00000	5157.80000	5157.90867	-0.00064

A22P-BR14KA153F-CC5HA 16

$$1/T = B0 + B1[LnRt] + B2[LnRt]^2 + B3[LnRt]^3$$

B0 = +9.300654648341D-04
B1 = +2.054846158443D-04
B2 = +5.738390888714D-06
B3 = -9.372019573911D-08

TABLE OF ERRORS

CALIBRATION TEMPERATURE DEG. C	CALIBRATION RESISTANCE OHMS	COMPUTED TEMPERATURE DEG. C	TEMPERATURE ERROR DEG. C
15.00000	22436.00000	15.00012	0.00012
25.00000	15041.00000	24.99951	-0.00049
37.00000	9591.60000	37.00085	0.00085
50.00000	6091.10000	49.99890	-0.00110
55.00000	5157.80000	55.00063	0.00063